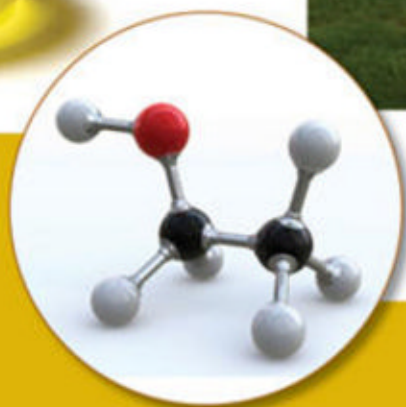


BIOFUELS from AGRICULTURAL WASTES and BYPRODUCTS



Hans P. Blaschek,
Thaddeus C. Ezeji &
Jürgen Scheffran

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**Hans P. Blaschek
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Edition first published 2010
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*Library of Congress Cataloging-in-Publication
Data*

Biofuels from agricultural wastes and byproducts / edited by Hans P. Blaschek, Thaddeus C. Ezeji, Jürgen Scheffran. – 1st ed.
p. cm.

Includes bibliographical references and index.
ISBN 978-0-8138-0252-7 (hardback : alk. paper) 1. Agricultural wastes as fuel.
2. Biomass energy. I. Blaschek, Hans P.
II. Ezeji, Thaddeus C. III. Scheffran, Jürgen.
TP360.B5745 2010
662'.88–dc22

2010011388

A catalog record for this book is available from the U.S. Library of Congress.

Set in 10/11.5 pt Times New Roman by Toppan
Best-set Premedia Limited
Printed in Singapore

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Preface

The demand for energy is rising and given that energy demand is projected to keep rising with constrained oil supplies, oil prices seem unlikely to fall significantly in the near future. Because 60% of U.S. petroleum supplies are imported, there is a need to develop alternative fuel supplies for future energy demands. Bioenergy has become a subject of increasing attention around the world. But the use of crop biomass such as grains, roots, and tubers as a raw material for bioenergy production may compete with food and feed supplies. U.S. fuel ethanol and biodiesel production is at an all-time high, but the industry is also facing a significant problem on how to deal with byproducts and wastes such as corn fiber, dried distillers' grains and solubles (DDGS), glycerin, food, and animal wastes. For instance, production of 10 lb of diesel results in 1 lb of glycerin and for every bushel of corn converted into ethanol (2.7 gallons), 18 lb of DDGS is generated. Waste, despite being one of the leading environmental problems, has the potential to become one of the largest bioenergy resources. Livestock production worldwide has grown rapidly in light of increased demand, and this has environmental implications especially in the area of waste management. In New York State alone, the dairy cow population is about 700,000, generating a significant amount of manure. At 40 lb of waste per cow per day, the energy potential is great. By eliminating the animal waste on a farm, a farmer alleviates or eliminates environmental problems, such as odor and water pollution, and may be able to increase the size of his herd. Animal waste digestion offers many economic benefits (biogas and fertilizer production). Therefore, finding new energy sources from livestock waste streams will be a major strategy to treat the waste and sustain the growth of the livestock industry.

Currently, there is no book on the market that is focused on the production of liquid biofuels and biogas from agricultural byproducts and wastes. This book will provide a comprehensive text on the science of production of liquid biofuels (ethanol and butanol) and biogas (methane) from agricultural byproducts as well as animal and food industry wastes. The book is intended for university researchers (professors, students, libraries), industry scientists (large company QA/QC management, bioenergy companies, start-up companies, microbiologists), as well as engineers and microbiologists from government agencies. This book should serve as an up-to-date reference resource for university and industry scientists in the area of biofuel research, waste treatment, and integrated farm management.

About the Editors

Hans P. Blaschek is a Professor of Microbiology and Director of the Center for Advanced BioEnergy Research (CABER) at the University of Illinois. He also serves as Assistant Dean of Biobased Research Initiatives in the Office of Research in the College of Agricultural, Consumer and Environmental Sciences, and is the theme leader of the Molecular Bio-engineering of Biomass Conversion Research Theme of the Institute for Genomic Biology. His research is focused on the acetone-butanol-ethanol fermentation and he is cofounder of a company called TetraVitae Biosciences that is currently commercializing the production of bio-butanol.

Thaddeus C. Ezeji received his PhD in Microbiology in 2001 from the University of Rostock Germany under the supervision of Prof. Dr. Hubert Bahl. He joined Dr. Hans Blaschek's laboratory at the University of Illinois Urbana-Champaign in 2001 as a postdoctoral research associate. Dr. Ezeji has been a faculty member of The Ohio State University since 2007, and his research has focused on fermentation, microbial strain development, metabolomics, and processes regulating the conversion of agricultural byproducts, coproducts, or wastes into biofuel and value-added products.

Jürgen Scheffran is a professor in climate change and security at KlimaCampus and the Institute for Geography of Hamburg University in Germany. Until summer 2009, he held positions at the University of Illinois at Urbana-Champaign (UIUC), in the Program in Arms Control, Disarmament and International Security, the Departments of Political Science and Atmospheric Sciences, and the Center for Advanced BioEnergy Research. After his physics PhD at Marburg University, he worked at Technical University of Darmstadt, the Potsdam Institute for Climate Impact Research. Recent activities include the Renewable Energy Initiative at UIUC and related projects funded by the Environmental Council, the Department of Energy, and the Energy Biosciences Institute.

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Biofuels from Agricultural Wastes and Byproducts

Chapter 1

Biofuels from Agricultural Wastes and Byproducts: An Introduction

Hans P. Blaschek, Thaddeus C. Ezeji, and Jürgen Scheffran

Around one-tenth of global primary energy use is based on bioenergy sources, of which about 10% are produced from modern bioenergy in the form of power, heat, and fuel. Biofuels for transportation account for 2.2% of all bioenergy, with a strong increase over the last decade. The total sustainable technical potential of bioenergy is estimated to be around a quarter of current global energy use.

Different from biomass specifically cultivated for energy purposes, residues and wastes are available as a byproduct of other processes. A significant amount of renewable energy is being generated from biogenic wastes and residues that do not require additional land and/or greenhouse gas emissions. Using their energy content would avoid methane emissions from slurry or landfills. Wastes and residues are quite heterogeneous: They arise in different sectors (agriculture and forestry, manufacturing, municipal enterprises, and private households) and at different stages of the value chain (biomass production and harvesting, processing, consumption, and disposal).

The technical potential of biogenic wastes and residues worldwide is estimated to be around 80 exajoules (EJ) per year. Research is needed in order to determine how much of this technical potential can be utilized in a sustainable and cost-effective way. A Department of Energy study has calculated in 2006 that over 1.3 billion dry tons per year of biomass from forestland and agricultural land alone are potentially available in the United States, a large fraction of which is from wastes and residues. This amount is sufficient to meet more than one-third of the current demand for transportation fuels while still meeting food, feed, and export demands. This biomass resource potential can be produced with relatively modest changes in land use, or agricultural and forestry practices.

Global production of biofuels in 2007 amounted to 16.4 billion gallons per year. Ethanol is currently the most important renewable liquid biofuel in the United States, which produces about half of the world's ethanol, compared with 38% in Brazil and 4.3% in the European Union. As the worldwide demand for fuels and chemicals surges and petroleum deposits are depleted, producers of ethanol fuel are increasingly looking beyond corn, potatoes, and other starchy crops as substrates for ethanol fuel production. Especially promising is cellulosic ethanol that can capitalize on microbial engineering and biotechnology to reduce costs.

Derived from low-cost and plentiful feedstocks, it can achieve high yields, has high octane, and other desirable fuel properties. Lignocellulosic feedstocks, such as switchgrass, woody plants, mixture of prairie grass, agricultural residues, and municipal waste, have been proposed to offer environmental and economic benefits. Compared to current biofuel sources, these biomass feedstocks require fewer agricultural inputs than annual crops and can be grown on agriculturally marginal lands.

After crop harvesting, the residues usually represent relatively large amounts of cellulosic material that could be returned to the soil for its future enrichment in carbon and nutrients or could be made available for further conversion to biofuels. Similarly, animal wastes are high in cellulose content and can also be converted to liquid biofuels. Such agricultural byproducts can play an important role in triggering the transition to sustainable biofuels.

Increasing demand for bioenergy has generated a strong interest in the bioconversion of agricultural wastes and coproducts into fuels and chemical feedstocks. To reduce the impact on land resources available for the production of food crops, a further increase in ethanol production will require the use of agricultural materials not directly tied to food, especially lignocellulosic biomass such as corn stover and corn fiber, wheat straw and rice straw, paper and wood processing waste, landscape waste and sugarcane waste. Some of the technologies to be utilized also generate coproducts such as electricity, hydrogen, ammonia, and methanol.

The chapters in this book cover a wide range of topics and demonstrate the potential for production of biofuels and chemicals from agricultural wastes and byproducts.

The chapter by *Nasib Qureshi, Stephen Hughes, and Thaddeus Ezeji* describes recent progress in emerging technologies to produce ethanol from lignocellulosic substrates, overcoming inhibitors generated during pretreatment, development of genetically improved cultures, simultaneous product recovery, and process integration. It addresses problems associated with inhibitor generation and detoxification, fermentation of both hexose and pentose sugars to ethanol, and the development of efficient microbial strains. Simultaneous product recovery, process consolidation, and integration will further improve the economics of production of biofuels from biomass. Coproducts serve as additional sources for generating revenue.

Fermentation of lignocellulosic biomass to ethanol requires additional processing steps for hydrolysis of biomass to simple sugars before these sugars can be fermented. These extra processing steps add to the overall cost of the substrate. Generally, the chemicals that are used to pretreat lignocellulosic substrates include dilute acid or alkali, and their use results in higher sugar yields when compared to pretreatments such as hot water or ammonia. These pretreatments generate products that inhibit cell growth and/or the fermentation process or both. Another challenging problem with respect to fermentation of biomass involves the inability of some fermentation microorganisms to use pentose sugars for growth and production of biofuels. Lignocellulosic biomass contains up to 30% pentose sugars, which are not utilized by the traditional ethanol-producing cultures such as *Saccharomyces cerevisiae*. Although recombinant cultures of *S. cerevisiae* have been developed, the overall productivity and ethanol concentration that can be achieved by these strains are not optimal.

Next-generation alternative renewable liquid biofuels are under development. Butanol can be used in internal combustion engines. It has higher energy content, is more miscible with diesel, is less corrosive, and has a lower vapor pressure and flash point than ethanol. Butanol can also be used at higher blend levels with gasoline or even at 100% concentration in car engines with little or no engine modification. Because of the solubility characteristics of butanol, it can be transported in existing fuel pipelines and tanks. Butanol can be produced by the fermentation route using renewable biomass. The low vapor pressure

of butanol facilitates its use in existing gasoline supply lines. As opposed to ethanol-producing cultures, butanol-producing cultures (e.g., *Clostridium acetobutylicum* or *Clostridium beijerinckii*) can use both hexose and pentose sugars released during hydrolysis of lignocellulosic biomass. During World War I and World War II, butanol plants existed worldwide, including those in the United States, the former Soviet Union (Russia), Canada, China, Japan, Australia, India, Brazil, Egypt, and Taiwan. As a result of various technological developments, attempts are being made to revive commercial production of butanol from agricultural residues for both chemical and biofuel use.

One of the major problems associated with bioproduction of butanol is the cost of substrate, which has led to recent interest in the production of butanol from alternative, inexpensive materials. However, much of the proposed alternative substrates, such as corn stover and fiber, wheat and rice straw, or dedicated energy crops such as switchgrass and *Miscanthus*, present challenges that need to be overcome before they can be used as commercial substrates for butanol production. The chapter by *Thaddeus Ezeji* and *Hans Blaschek* details the butanol pathway, including pretreatment and hydrolysis; generation of lignocellulosic degradation products; effects of degradation products on growth and butanol production by fermenting microorganisms; and strategies for improved utilization of lignocellulosic hydrolysates.

Most bacteria use glucose as a preferred carbon source for growth, and only when glucose is limiting are the pentose sugars utilized, making fermentation of complex mixture of sugars in lignocellulosic hydrolysates challenging. The solventogenic acetone-butanol-ethanol (ABE)-producing clostridia have an added advantage over many other cultures in that they can utilize both hexose and pentose sugars, which are released from wood and agricultural residues upon hydrolysis in order to produce ABE. Pretreatment can result in the formation of a complex mixture of microbial inhibitors that are detrimental to growth of fermenting microorganisms. Options for the reduction or elimination of lignocellulosic degradation products during pretreatment include the removal of inhibitors prior to fermentation, development of inhibitor-tolerant mutants, or a combination of the above approaches. The development of inhibitor-tolerant mutants via culture adaptation appears to be the most viable approach from an economic standpoint, a research area in which the authors are currently involved.

Largus T. Angenent and *Norman R. Scott* discuss practical aspects and future directions for methane production from agricultural wastes. Anaerobic digestion is a proven technology for bioconversion of agricultural waste that is high in organic material to gaseous biofuel. It provides an efficient energy recovery system because methane and carbon dioxide are automatically and constantly removed from the process by degassing (bubble formation). Intermediate products in the food chain are converted into methane with very low concentrations of carboxylic acids in the digester effluent and hydrogen in the off gas. While methane formation is a remarkable conversion process that circumvents product inhibition, it is susceptible to instabilities. This chapter discusses the anaerobic digestion of mixed cultures in which the waste material can be complex and variable in composition over the operating period. Practical studies assess the performance, stability, and limitations of methane fermentation, and the economic or environmental benefits in the conversion of agricultural residues.

In addition, upgrading the energy carrier methane to more valuable products may be necessary to guarantee economical viability. With the need for co-digestion, an opportunity exists to link agriculture, rural communities, and industry for sustainable rural community development, providing a number of specific case studies. A U.S. example for this system approach is BioTown in Richmond, Indiana, where the goal is to create an energy self-sufficient

community using an anaerobic digester as an integrated technology to create biogas from animal manures, food wastes, organic municipal wastes, and crop residues. Communities such as these illustrate that anaerobic digestion is a significant and working technology with relatively high-energy efficiencies and that agricultural wastes play an essential role in such systems. Anaerobic digestion of agricultural wastes is a mature technology with numerous full-scale digesters all over the world. Even though the level of maturity is high, research on reactor stability is necessary. More powerful techniques, such as metagenomics and stable-isotope probing, are starting to shed light on our understanding of anaerobic digestion. For wastes from agriculture with complex nutrient and water cycles, anaerobic digestion should be seen in the larger context of an integrated system in which nutrients and water from digester effluent are continuously recycled.

The chapter by *Edward A. Bayer, Raphael Lamed, Bryan A. White, Shi-You Ding, and Michael E. Himmel* addresses the current status of knowledge regarding the function of cellulases and cellulosomes, and how they might be used in biomass conversion to biofuels. This includes a description of various types of cellulosic biomass in agricultural wastes and the pretreatment strategies required to enhance enzymatic hydrolysis and to avoid toxic by-products that would interfere with enzyme action and fermentation. The search for novel enzymes, and strategies for mutation and modification of cellulases and cellulosomes for future application to bioenergy initiatives are considered as well. Some of the bottlenecks and pitfalls in providing efficient processes for conversion of cellulosic biomass to fermentable sugars for biofuels production are addressed. To develop successful future bioconversion processes, it is promising to mimic the concerted action of the cellulolytic microbes, the bacteria, and fungi that have evolved to produce cellulases and cellulosomes.

Structural biomass is a rich and renewable source of fermentable sugars for industrial production of biofuels. In attempting to utilize polysaccharides in lignocellulosic carbohydrates at the commodity scale, one must consider a key principle set forth in the evolutionary development of the cell wall of terrestrial plants, namely essential recalcitrance to deconstruction. The major bottleneck in this process is the deconstruction of the plant cell wall, liberating both C6 and C5 sugars. Nature has evolved microbes and their enzymes to deal primarily with damaged and decaying vegetation. Progress is being made in this endeavor, although the key cost challenges remain the subject of considerable international research focus today. New and improved enzyme systems closely coupled to related process technologies, such as biomass pretreatment, are required to provide cost-effective and large-scale quantities of liquid fuels from biomass.

Syed Shams Yazdani, Anu Jose Mattam, and Ramon Gonzalez describe the production of fuel and chemicals from glycerin (or glycerol) that is generated as an inevitable byproduct during the biodiesel production process, as well as at oleochemical and bioethanol production plants. Due to the tremendous growth in the biofuels industry, glycerin is now regarded as a waste product that needs to be disposed at a cost. Glycerol is not only abundant and inexpensive, but also offers the opportunity to produce fuels and reduced chemicals at yields higher than those obtained with the use of common sugars. Anaerobic fermentation converts abundant and low-priced glycerol streams generated in the production of biodiesel into higher-value products and represents a promising route to achieving economic viability in the biofuel industry. A number of organisms are able to ferment glycerol and synthesize products with a wide range of functionalities. There are many advantages for the use of glycerol over sugars, which together translate into lower capital and operational costs.

As the chapter shows, there are few organisms that are able to utilize glycerol in the absence of external electron acceptors and that produce high-value chemicals such as 1,3-propanediol,

succinic acid, propionic acid, and biosurfactant. In their recent studies, the authors have discovered that *Escherichia coli* can fermentatively metabolize glycerol and have established the pathways, mechanisms, and conditions enabling this metabolic process. The knowledge base created by the authors has opened up a new platform to engineer *E. coli* for the production of several fuels and chemicals, including the production of ethanol along with coproducts hydrogen and formate at high yields and productivities.

Commercial scale utilization of lignocellulosic biomass is not a trivial task and is quite different from the use of grain. As Klein E. Ileleji, Shahab A. Sokhansanj, and John S. Cundiff show, farm-gate to plant-gate delivery of lignocellulosic feedstocks from plant biomass for biofuel production is a key cost factor. The logistics and handling cost of feedstock can be very expensive and is one of the major reasons for the high cost of producing liquid fuels and power from lignocellulosic feedstocks. While diverse types of biomass may be chemically similar, they are quite different with respect to their times of harvest/collection and physical characteristics. This means the unique differences of these feedstocks need to be considered when designing an effective biomass logistics system. Harvest is followed by transportation to on-farm storage, preprocessing, or biorefinery plants. Sustainable supply of feedstock from on-farm storage must be delivered to the biorefinery plant year round. To minimize costs, the design, operation, and coordination of efficient feedstock delivery systems is vital.

The chapter compares three herbaceous biomass logistic systems (cotton, sugarcane, and grain) with a woody biomass system (fuel chips) and explores the linkage between harvesting, in-field hauling, and over-the-road hauling. In all short-haul systems, truck productivity is maximized when the load time and unload time is minimized. Given the variability of field conditions, logistic systems must provide for efficient flow of material into and out of at-plant storage, which is critically important for any plant that has a high cost penalty for shutdown. Typically, the feedstock cost constitutes one-third to one-half of the total production cost of ethanol or power, where the actual percentage depends upon biomass species, yield, location, climate, local economy, and the type of systems used for harvesting, gathering and packaging, processing, storing, and transporting of biomass as a feedstock.

A logistic system for forage chopping has basically the same challenges as the sugarcane system, but as the authors note, it is not practical in the United States to have several hundred farmers chopping biomass and delivering on their own schedule to the bioenergy plant. Baling provides a disconnect between the harvest and in-field operations, which is a significant advantage. One operator can bale an entire field with no requirement to coordinate with any other operation. A systems integration approach is increasingly important to design systems and analyze pathways along the whole supply chain, including harvest, storage, and transport. A systems approach examines the complete system to see what processes can be combined for synergy of resources, reduction in waste, and cost reduction. An integrated approach seeks to combine multiple tasks, for instance, by harvesting grain and fiber together and subsequently separating them. Systems integration also helps to overcome bottlenecks and lack of close collaboration, which allows everyone to see the bigger picture of how everything fits in place and harness the optimal strengths of the complex production chain as a whole.

Numerous opportunities exist to make ethanol plants more productive by reducing waste and expanding the diversity of both inputs and outputs. According to Timothy C. Lindsey's assessment, byproducts and wastes from other industries such as food processing, landscaping, paper, and municipal solid waste facilities could be substituted for crops as feedstocks and processed into ethanol and other high-value products, thereby reducing the strain on food resources. A wide variety of cellulose-based biomass wastes and byproducts is available

for conversion to biofuels, including agricultural residues (corn stalks and cobs, straws, cotton gin trash, and palm oil wastes); paper (paper mill sludge, recycled newspaper, and sorted municipal solid waste); wood waste (sawdust, wood chips, and prunings); and landscape waste (leaves, grass clippings, and vegetable and fruit wastes). Most of these materials are available at very low cost and some even command tipping fees associated with their disposal as wastes. This chapter points out that the United States currently converts approximately 15 million tons of agricultural products into ethanol and biodiesel, and discards approximately 270 million tons of agriculturally derived residues in the form of harvestable crop residues, animal manure, forest residues, and the organic fraction of municipal solid wastes.

This contribution describes two incremental opportunities and modifications for implementing existing technology that would enable expansion of existing dry-mill ethanol operations to biorefineries with respect to feedstocks and products. Modification of existing processes to incorporate cellulosic feedstocks into existing operations could greatly improve the diversity and flexibility of feedstock options. Recovery of oil from byproducts such as germ, thin stillage syrup, or dried distillers' grains and solubles (DDGS) could expand greatly the quantities and value of products produced from dry-mill plants and also provide valuable feedstock options for biodiesel producers. The DDGS could be further fractionated to separate and pelletize high-protein/high-value components from lower-value materials.

Furthermore, cogeneration systems could be implemented to burn lignin and other coproducts to simultaneously produce steam and electricity, thereby reducing requirements from external sources and providing electrical power for additional biorefining operations. Ethanol is an important industrial ingredient and has widespread use as a base chemical for other organic compounds (e.g., ethyl halides, ethyl esters, diethyl ether, acetic acid, butadiene, and ethyl amines) that could provide unique opportunities for expanding operations in the future.

Emphasizing that cellulosic biomass is an inexpensive and abundant resource to collect and store large-scale solar energy, *Bin Yang, Yanpin Lu, and Charles E. Wyman* demonstrate that agricultural residues are particularly promising for initial commercial applications because of their potential low cost and near-term availability. Because feedstock costs are dominant in processing economics, it is critical to seek those for first applications that are low cost and sufficiently abundant. High product yields and ease of processing are also vital to minimizing costs, while sufficient amounts must be available to achieve reasonable economies of scale. Agricultural residues are expected to serve as a major biofuels feedstock, and their potential low cost and current availability can be particularly important in the near term.

The rapidly evolving tools of biotechnology can significantly lower conversion costs and enhance yields, making biological processing a particularly promising approach to converting these solids into liquid fuels and chemicals and providing environmental, economic, and strategic benefits. The most expensive processing step is the breakdown of cellulosic materials into cellulose and hemicellulose to release fermentable sugars, while pretreatment has pervasive impacts on all other major operations.

This chapter summarizes estimated amounts of major agricultural residues and their potential for making ethanol, including environmental factors that determine their availability, composition, and reported yields. Emphasis is given to approaches, needs, and costs for harvesting, transporting, and storing agricultural residues with only little degradation of the feedstock. The economics of processing residues to ethanol demonstrates the importance of feedstock composition, availability, and cost to good returns on capital. Finally, opportunities and strategies for advanced technologies to lower the cost of biological processing to ethanol and other products are outlined. In estimating the potential for large-scale fuel production,

consideration must be given to how much can be removed without negative environmental consequences such as depletion of soil carbon and soil erosion.

Selection of low-cost residues can be particularly important for overcoming the many obstacles to implementation of cellulosic ethanol technology. According to the authors, these include perceived risks and the associated high rates of return on capital, overdesign to compensate for risk, suboptimal facility sizes that keep investment costs lower but fail to capitalize on economies of scale, and other disadvantageous burdens. Taking advantage of other economic levers such as integration of production of valuable coproducts from lignin, minerals, or other components into an existing fermentation or power facility to reduce capital costs, and use of low-cost debt financing through partnerships with municipalities or others can have a tremendous impact on commercial success. Government assistance could prevent the private sector from bearing huge investments and make sure that projects are economically viable. Once in place, significant learning curve improvements and technology advances would lower costs to make them competitive without government support.

Yuanhui Zhang analyzes thermochemical conversion (TCC) processes of biomass, including gasification (e.g., Fischer–Tropsch process), direct liquefaction, hydrothermal process, and pyrolysis. TCC is a chemical reforming process of biomass in a heated and usually pressurized, oxygen-deprived enclosure, where long-chain organic compounds (solid biomass) break into short-chain hydrocarbons such as syngas or oil. Gasification of biomass produces syngas, a mixture of hydrogen and carbon monoxide that is then reformed into liquid oil with the presence of a catalyst. Pyrolysis is a heating process for converting dried biomass to syngas and oil in the absence of oxygen.

Hydrothermal processes (HTP) of various biomass feedstocks, including biowaste (manure and food processing waste), lignocellulose (crop residue), and algae, involves direct liquefaction of biomass, with the presence of water and perhaps some catalysts, to directly convert biomass into liquid oil. HTP mimics the natural process of fossil fuel formation from biomass feedstocks and holds great promise as a renewable energy technology, especially for liquid fuel. Converting biowaste materials into liquid fuel through HTP holds several unique advantages. Using biowaste has a net-zero carbon emission and provides a negative-cost feedstock that does not compete with the food supply.

During the deconstruction process, the natural protection mechanisms of biological systems to foreign intrusions have created tremendous obstacles for the economical production of biofuels. It is precisely for this reason why a large-scale production of biofuel from renewable sources represented by biomass is still a challenge to the scientific and engineering communities. As *Bin Wang* and *Hao Feng* describe, among the restrictive factors is the generation of inhibitory chemicals during the deconstruction process, with either a chemical, enzymatic, or biological process. Inhibitory compounds will reduce the solvent production ability of a fermenting organism, or even totally block the growth and metabolism of the cells. A detoxification step may be used to remove the inhibitors or mitigate their negative effect before fermentation, based on chemical, physical, or biological methods. In this chapter, the technical aspects of the detoxification approaches are discussed with a focus on alternative detoxification methods as well as their potential applications in biomass-to-fuel production.

Chapter 2

Production of Liquid Biofuels from Biomass: Emerging Technologies

Nasib Qureshi, Stephen Hughes, and Thaddeus C. Ezeji

Abstract

This is an overview of the emerging technologies that have been developed recently or are in the process of development for ethanol (biofuel) production from agricultural residues. In this direction numerous advances have been made. Problems associated with inhibitor generation and detoxification, fermentation of both hexoses and pentoses to ethanol, and development of efficient microbial strains have partially been addressed. Simultaneous product recovery and process consolidation and/or integration will further improve the economics of production of biofuels from biomass. It is emphasized that numerous domestic and international companies have initiated their programs to commercialize conversion of biomass (agricultural residues) to biofuels. Separation and use of byproducts as additional sources of generating revenues can strengthen this fermentation further.

Introduction

Traditional substrates that have been used to produce biofuels such as ethanol and butanol include molasses, corn, and whey permeate. Molasses is a byproduct of the sugarcane-processing industry and contains approximately 50% sugar. This substrate (molasses) has been used to produce ethanol in countries such as Australia, Brazil, and India. Corn has been used in the United States, while whey permeate has been used in New Zealand. The use of these substrates affects the economics of biofuel production (Qureshi and Manderson 1995) and is becoming more challenging due to these substrates' use for food and feed. Currently about 140 billion gallons of gasoline is used in the United States annually, of which 15% can be supplemented by corn ethanol (Qureshi and Ezeji 2008). In order to reach a higher level of supplementation, lignocellulosic biomass such as corn stover, wheat straw, barley straw, switchgrass, and reed canary grass has to be used.

Fermentation of lignocellulosic biomass to ethanol requires additional processing steps for the hydrolysis of biomass to simple sugars before these sugars can be fermented. These extra processing steps add to the overall cost of the substrate. Generally, the chemicals that are used to pretreat lignocellulosic substrates include dilute acid (H_2SO_4), or alkali (NaOH), and their use results in higher sugar yields when compared with pretreatments such as hot water or ammonia. These pretreatments (acid/alkali) generate products that inhibit cell growth and/or the fermentation process or both. Another challenging problem with respect to fermentation of biomass involves the difficulty by various fermentation microbes for using pentose sugars. Lignocellulosic biomass contains up to 30% pentose sugars, which are not utilized by the traditional ethanol-producing cultures, such as *Saccharomyces cerevisiae*. During the last two to three decades new cultures of *Saccharomyces cerevisiae* that can utilize both hexose and pentose sugars released from lignocellulosic hydrolysates have been developed (Hahn-Hägerdal and Pamment 2004; Sedlak and Ho 2004; Hughes et al. 2009a,b). Although such cultures have been developed, the overall productivity and ethanol concentration that can be achieved by these strains are not optimal. This chapter describes recent progress with respect to the development of emerging technologies that have been developed to produce ethanol from lignocellulosic substrates. These challenging technologies include use of lignocellulosic biomass substrates, overcoming (at least partially) inhibitors generated during the pretreatment process, development of genetically improved cultures, simultaneous product recovery technologies, and process integration.

Butanol can be used in internal combustion engines. This biofuel can be produced by the fermentation route using renewable biomass (Ezeji et al. 2007a,b). Compared with ethanol, butanol is less volatile, less sensitive to water, less flammable, and has a slightly higher octane number. Its low vapor pressure facilitates its use in existing gasoline supply lines. As opposed to ethanol-producing cultures, butanol-producing cultures (*Clostridium acetobutylicum* or *Clostridium beijerinckii*) can use both hexose and pentose sugars released during hydrolysis of cellulosic biomass. During World War I and II there were plants worldwide including those in United States, the former Soviet Union (Russia), Canada, China, Japan, Australia, India, Brazil, Egypt, and Taiwan. As a result of various technological developments, attempts are being made to revive commercial production of butanol from agricultural residues. Details of this fermentation are presented in Chapter 3.

Economically Available Agricultural Residues as Substrates

As a result of increasing gasoline prices, the use of ethanol as a biofuel has been introduced on a large scale in Brazil, the United States, and various European nations. As stated in the Introduction section, it is essential to use lignocellulosic biomass in order to meet the global biofuel demand. Prices of agricultural residues and energy crops are much lower than those of corn (wheat straw \$24, barley straw \$26, corn stover \$50, grass hay \$50, and switchgrass \$60/ton). In the past few months corn prices have reached \$230/ton. While agricultural residue substrates are available at a much lower cost, their processing faces a number of challenges before they can be converted to ethanol. These challenges include (1) pretreatment and enzymatic hydrolysis of lignocellulosic biomass to monomeric sugars; (2) generation of fermentation inhibitors during pretreatment; and (3) fermentation of mixed sugars (hexoses and pentoses) to ethanol. Pretreatment of agricultural residues or energy crops is essential to soften the fiber structure and make it available for enzymatic action and hence for further

hydrolysis. Although there are numerous pretreatment technologies available, including dilute sulfuric acid, sodium hydroxide, steam expansion, and ammonia treatment, dilute sulfuric acid treatment still offers higher sugar yield than other methods. Pretreated and hydrolyzed biomass solutions contain hexoses (e.g., glucose, galactose, and mannose) and pentoses (e.g., xylose and arabinose). Fermentation of hexoses can be achieved efficiently using traditional strains such as *S. cerevisiae* and *Zymomonas mobilis*. However, the fermentation of pentose sugars poses problems when using parental microbial strains and will require engineering of these traditional strains with new metabolic pathways.

Fermentation Inhibitors

Pretreatment of lignocellulosic biomass using dilute sulfuric acid treatment is carried out at high temperature (121°C for 1 hour). During this process some of the sugars released from biomass react and form chemicals that are inhibitory for cell growth or fermentation, or both. *Pichia stipitis*, a natural pentose-fermenting yeast, is inhibited by compounds produced during pretreatment and hydrolysis of lignocellulosic biomass (Slininger et al. 2009). Examples of some of these inhibitors include furfural, hydroxymethyl furfural, acids (acetic, ferulic, glucuronic, vanillic, syringic, and *p*-coumaric), and other chemicals such as vanillin and syringaldehyde (Tran and Chambers 1985; Grohmann and Bothast 1997; Ezeji et al. 2007a). To be able to ferment toxic hydrolysates to ethanol or butanol a number of detoxifying methods exist such as treatment with $\text{Ca}(\text{OH})_2$ (also called overliming), use of XAD resins (Sigma Chemicals, St. Louis, MO), or use of cultures that can metabolize the inhibitors. Studies on developing such inhibitor-metabolizing strains (*Coniochaeta ligniaria*) have been successful (Nichols et al. 2008). However, inhibitor utilization has to be carried out before the production of ethanol when using hexose- and pentose-fermenting strains. Another alternate could be the development of cultures that can tolerate and utilize inhibitors and still produce ethanol or butanol. In an interesting approach, acidic and alkaline-electrolyzed water was used to hydrolyze agricultural biomass and produce biofuel without any significant inhibitory effects on the microbial cultures (Wang et al. 2009). However, a much lower sugar yield was obtained when employing these approaches than when using dilute H_2SO_4 .

Genetically Engineered Cultures

In traditional ethanol fermentations *S. cerevisiae* has been used to produce this biofuel from either sugarcane or corn. These substrates contain glucose or sucrose (a disaccharide of glucose and fructose) and do not contain pentose sugars. Although a natural xylose-fermenting yeast, *Pichia stipitis* produces ethanol with comparatively good productivity (Hahn-Hägerdal et al. 2006) from pentose sugars; it is inhibited when biomass hydrolysates are employed. For this and other reasons, an ideal ethanol-producing culture should possess the following characteristics: should not be inhibited by inhibitors produced during biomass pretreatment as well as its own metabolic byproducts such as acetic and lactic acids; should be able to utilize hexose and pentose sugars; and should be able to produce and tolerate a high concentration of ethanol. Production of byproducts such as acetic acid reduces ethanol yield and arrests cell growth and the fermentation process. A large number of studies are

Table 2.1. Production of ethanol from xylose or mixed sugars using genetically modified cultures to use pentose sugar/s.

Culture	Maximum Ethanol Concentration (g/L)	Ethanol Yield (g/g)	Productivity (g/L.h) or Specific Productivity (g/g Cell.h)	References
<i>Escherichia coli</i> KO11	40.9	0.47	0.38 g/g.h	Hahn-Hägerdal and Pamment 2004
<i>E. coli</i> FBR5	43.5	0.50	0.90 g/L.h	Qureshi et al. 2006
<i>Klebsiella oxytoca</i> M5A1(pLOI555)	46.0	0.48	0.96 g/L.h	Dien et al. 2003
<i>Pichia stipitis</i> NRRL Y-7124	22.3	0.43	0.47 g/L.h	Nigam 2001
<i>Zymomonas mobilis</i> 8b	54.0	0.47	1.13 g/g.h	Mohaghegi et al. 2004
<i>Saccharomyces cerevisiae</i> TMB 3006	—	0.37	0.66 g/g.h	Hahn-Hägerdal and Pamment 2004
<i>S. cerevisiae</i> TMB 3400	—	0.25	0.10 g/g.h	Hahn-Hägerdal and Pamment 2004
<i>S. cerevisiae</i> TMB 424A(LNF-ST)	46.5	0.43	—	Sedlak and Ho 2004

—, information not available.

being focused toward this direction with the aim of developing suitable microbial strains; however, only limited success has been achieved to date. The recombinant strains of yeast and bacteria that have been developed include *Escherichia coli* (KO11 and FBR5; Ingram et al. 1987), *Klebsiella oxytoca*, *Zymomonas mobilis*, and *S. cerevisiae* (424A [LNF-ST], TMB3006, TMB3400). So far only recombinant *S. cerevisiae* strains have been able to produce ethanol from xylose contained in non-detoxified hydrolysates. Some of these *S. cerevisiae* strains have been used in fed-batch systems in combination with extremely inhibitory softwood hydrolysates (Hahn-Hägerdal et al. 2006). Details of some of the fermentation parameters that resulted from the use of these xylose-utilizing strains have been listed in Table 2.1.

Simultaneous Product Removal Techniques

As most recombinant strains cannot tolerate high concentrations of ethanol, an alternative solution to this problem could be the simultaneous removal of ethanol as it is produced. As ethanol is removed from the fermentation broth, the culture is relieved of the ethanol toxicity effects and as a result produces more ethanol. As ethanol is produced, the level of sugar present in the reactor is reduced. To replace sugar, the reactor is fed with a concentrated sugar solution at a rate that is compatible to sugar utilization by the microbe for ethanol production. This process is called fed-batch and is applicable to systems where the use of a concentrated sugar solution is toxic to the culture. It should be noted that most lignocellulosic hydrolysates contain low sugar levels, hence a combination of lignocellulosic hydrolysate supplemented with glucose to raise the sugar level is an approach to consider. Alternately, cultures that are

not inhibited by high sugar concentrations can be used in batch systems with simultaneous product recovery. In such systems all the sugars present in the bioreactor are converted to a final product. Application of these simultaneous product recovery techniques would reduce reactor size, process stream volume, and result in energy-efficient removal of final product. Use of these techniques for butanol fermentation has been successfully demonstrated in laboratory scale reactors (Qureshi 2009). Two of the most common technologies that can be used for product recovery include gas stripping and pervaporation. Gas stripping can be performed using fermentation gases (CO_2 in the case of ethanol production, and CO_2 plus H_2 in the case of the butanol fermentation). The use of pervaporation requires a selective membrane that allows diffusion of biofuel only and restricts water transport across the membrane.

Consolidated or Integrated Fermentation

Process integration results in the reduction of capital and operational costs. There are processes known as separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and simultaneous saccharification fermentation and recovery (SSFR). In SHF both hydrolysis and fermentation are carried out in separate reactors. In SSF the enzymatic hydrolysis continuously releases sugars that are used by the culture simultaneously. The process integrated with product recovery is called SSFR. SSF can also be achieved by performing both hydrolysis and fermentation in a single bioreactor employing single or mixed microbial strains that produce all the necessary enzymes and use all the hexose and pentose sugars. This type of process is called consolidated/integrated bioprocessing (CBP/IBP; Lynd et al. 2005). The CBP/IBP differs from SSF because CBP does not require addition of exogenous hydrolytic enzymes (the ethanol-producing microbial strain produces them), unlike SSF, which requires addition of exogenous enzymes for substrate hydrolysis. These processes (CBP/IBP) can be integrated with simultaneous product recovery that can be called CBP/IBP with product recovery (CBPPR or IBPPR).

Cellulosic Biorefineries for Ethanol/Butanol Production

Companies in the business of using biomass sugars for fermentation can rely on a sustainable source of low-cost material. This alternative source of sugars can be exploited to fill the growing demand for transportation biofuels to supplement the need for crude oil and secure a domestic (and international) supply of liquid transportation fuel. The cellulosic microorganisms can provide a platform in which to make other beneficial proteins and products for the developing cellulosic ethanol industry. Existing corn starch in ethanol facilities provide locations into which pilot lignocellulosic biorefineries can be bolted. This alternative biomass feedstock is expected to provide a great resource for cellulosic fermentation to ethanol.

Ethanol produced from cellulosic biomass has the potential as a large-scale transportation fuel. Desirable features include ethanol's fuel properties as well as benefits with respect to air quality, global climate change, balance of trade, and energy security. Energy balance, feedstock supply, and environmental impact considerations are not seen as significant obstacles to the widespread use of fuel ethanol derived from cellulosic biomass. Profitability of the conversion is the major challenge, however.

Biomass is the only known, large-scale, renewable resource that can be converted into liquid fuels for transportation. Cellulosic ethanol is particularly promising because it can capitalize on microbial engineering and the power of biotechnology to reduce costs, is derived from low-cost and plentiful feedstocks, can achieve high yields, has high octane and other desirable fuel properties, and is environmentally friendly. Lignocellulosic feedstocks, such as switchgrass, woody plants, mixtures of prairie grasses, agricultural residues, and municipal waste, have been proposed to offer environmental and economic advantages over current biofuel sources, because these biomass feedstocks require fewer agricultural inputs than annual crops and can be grown on agriculturally marginal lands.

As a result of the above-mentioned advantages and developments made with respect to the production of liquid biofuels from cellulosic biomass, numerous companies have initiated programs to use this substrate and commercialize ethanol or butanol production. The companies that have initiated programs on production of biofuels from biomass in the United States include Coskata (Warrenville, IL), Poet Inc. (Soiux City, SD), RangeFuels (Broomfield, CO), Amyris (Emeryville, CA), Mascoma (New Hampshire), the DuPont Danisco BP venture (Wilmington, DE), BlueFire Ethanol (Irvine, CA), Qteros (Marlborough, MA), Verenium (Cambridge, MA), Valero (Sioux Falls, SD), ExxonMobil and Synthetic Genomics, Inc. (LaJolla, CA), KL Energy (Rapid City, SD), INEOS (Fayetteville, AR), Osage Bioenergy, Inc. (Hopewell, VA), Cobalt Biofuels (Mountain View, CA), Tetravita Biosciences (Chicago, IL), and Gevo (Colorado). There are numerous other international companies that have started working on the production of biofuels from biomass, including Petrobras (Sao Paulo, Brazil), Iogen (Ottawa, Ontario, Canada), SEKAB & Taurus Energy (Örnsköldsvik, Sweden), Abengoa Bioenergy (Salamanca, Spain), and Praj Industries (Pune, India).

Conclusions

In conclusion, numerous advances have been made in the conversion of biomass to biofuels as biomass is the only source that is renewable and economically available. Problems associated with inhibitor generation and detoxification, fermentation of both hexoses and pentoses to ethanol, and the development of efficient microbial strains have partially been addressed. Simultaneous product recovery and process consolidation and integration will further improve the economics of production of biofuels from biomass. It is emphasized that numerous domestic and international companies have initiated their programs to commercialize conversion of biomass to biofuels. Separation and use of coproducts as additional sources for generating additional revenue can strengthen the approach further.¹

Acknowledgements

Nasib Qureshi would like to thank Adam Wallenfang for his help on finding information on published data. Nasib Qureshi and Stephen Hughes would like to thank Michael A. Cotta (U.S. Department of Agriculture, Peoria, IL) for reading this manuscript and providing critical comments.

Endnote

1. Mention of trade names or commercial products in this article is solely for the purpose of providing scientific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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Chapter 3

Butanol Production from Lignocellulosic Biomass

Thaddeus C. Ezeji and Hans P. Blaschek

Abstract

As the worldwide demand for fuels and chemicals surges and petroleum deposits are depleted, producers of ethanol fuel are increasingly looking beyond corn, potatoes, and other starchy crops for substrates for ethanol fuel production. Ethanol is currently the most important renewable liquid biofuel, but it has problems ranging from lesser energy content than gasoline, blending limitations with gasoline, potential for corrosion of pipes, and subsequent inability to be transported using existing pipeline infrastructure, to requiring modification of car engines with increasing ethanol concentrations such as E85 or 100% ethanol. Attempts are underway to produce alternative renewable liquid biofuels and chemical feedstocks that are superior to ethanol. Butanol is one such biofuel because it has greater energy content, is more miscible with diesel, is less corrosive, and has a lesser vapor pressure and flash point than does ethanol. Butanol can also be used at greater blend amounts with gasoline or even at 100% concentration in car engines with little or no engine modification, and because of its solubility characteristics, it can be transported in existing fuel pipelines and tanks. One of the major problems associated with bio-based production of butanol is the cost of substrate. The cost of substrate has led to recent interest in the production of butanol from alternative, inexpensive materials. However, much of the proposed alternative substrates, such as corn stover, corn fiber, wheat straw, rice straw, or dedicated energy crops such as switchgrass and *Miscanthus*, present challenges that need to be overcome before they can be used as commercial substrates for butanol production. This chapter, therefore, details the (1) pretreatment and hydrolysis of various lignocellulosic biomass; (2) generation of lignocellulosic degradation products during pretreatment of biomass; (3) effects of degradation products on growth and butanol production by fermenting microorganisms; and (4) strategies for improving lignocellulosic hydrolysates utilization for butanol production.

Introduction

Increasing energy demand worldwide coupled with a limited supply of fossil fuels and the fluctuating price of oil has generated a strong interest in the bioconversion of agricultural biomass and coproducts into fuels and chemical feedstocks. Biofuels are currently produced mainly from carbohydrates (corn, potato, sugar cane, sugar beets, etc.) and oil (soybean, rapeseed, etc.)-rich crops. Ethanol was once considered to be a replacement for gasoline, a fossil fuel currently used in car engines. A study conducted by the U.S. Department of Agriculture (USDA) in 2007 projected that the average corn price will peak at \$3.75 per bushel during the 2009–2010 marketing year and will decline before stabilizing at approximately \$3.30 through 2016 (USDA-ERS 2007). An expansion of the ethanol and biodiesel industry has influenced the prices of corn and soybeans in the United States. In 2008, approximately 9.0 billion gallons of ethanol was produced from corn in the United States for use as a fuel supplement (Renewable Fuel Association 2008), which represents approximately 4.2% of total gasoline consumption (150 billion gallons; 1 gallon ethanol = 0.7 gallons of gasoline). An increase in ethanol production from corn would require additional acreage and would potentially have an impact on land available for the production of food crops. Therefore, a further increase in ethanol production will require the use of agricultural materials not directly tied to food, especially lignocellulosic biomass such as corn stover, corn fiber, wheat straw, rice straw, or other energy crops such as switchgrass and *Miscanthus*. Lignocellulosic biomass, which may contain xylan, arabinan, galactan, glucuronic, acetic, ferulic, and coumaric acids, is the most abundant renewable resource on the planet (Koukiekolo et al. 2005) and has great potential as a substrate for butanol production (Ezeji et al. 2007a,b).

Butanol is a four-carbon alcohol with some very interesting attributes with respect to its use as a fuel and fuel extender that are greater than ethanol (Ezeji et al. 2004b; Ezeji and Blaschek 2007). Prior to 1950, the AB (acetone butanol) or acetone butanol ethanol (ABE) fermentation using corn and molasses as substrates, ranked second only to the ethanol fermentation in its importance and scale of production, but subsequently declined due to increasing substrate (sugars and molasses) costs and availability of much cheaper petrochemically derived butanol (Ezeji and Blaschek 2007; Schwarz et al. 2007). Substrate cost has long been recognized as having a dramatic influence on butanol price (Qureshi and Blaschek 2000). Most bacteria use glucose as a preferred carbon source for growth, and only when glucose is limiting are the pentose sugars utilized, making fermentation of complex mixture of sugars in lignocellulosic hydrolysates challenging (Ezeji et al. 2007a). The solventogenic ABE-producing clostridia have an added advantage over many other cultures in that they can utilize both hexose and pentose sugars, which are released from wood and agricultural residues upon hydrolysis to produce ABE (Ezeji et al. 2007b).

The solvent-producing clostridia are not able to hydrolyze fiber-rich agricultural residues or lignocellulosic biomass. The lignocellulosic biomass must be pretreated and hydrolyzed to simple sugars using physical (size reduction), chemical, and enzymatic methods. Unfortunately, these treatments can result in the formation of a complex mixture of microbial inhibitors that are detrimental to the growth of fermenting microorganisms (Ezeji and Blaschek 2008a). Examples of inhibitory compounds produced include furfural, hydroxymethylfurfural (HMF), syringaldehyde, and acetic, ferulic, glucuronic, *p*-coumaric, syringic, levulinic acids, and so on (Zaldivar et al. 1999; Zaldivar and Ingram 1999; Varga et al. 2004; Ezeji et al. 2007b). The reduction or elimination of lignocellulosic degradation products during the

pretreatment of biomass, removal of inhibitors from lignocellulosic hydrolysates prior to fermentation, adaptation of strains (to these inhibitors) via the development of inhibitor tolerant mutants, or a combination of the above approaches have been touted to be the panacea for successful production of biofuels from lignocellulosic biomass. Among these options, the development of inhibitor-tolerant mutants via culture adaptation appears to be most viable approach from an economic standpoint. Many laboratories, including those of the authors, are currently involved in research directed toward development of inhibitor-tolerant butanol-producing microorganisms.

Butanol Production from Lignocellulosic Biomass

Composition of Lignocellulosic Biomass and Hydrolysates

Lignocellulosic biomass is composed of mainly cellulose, hemicellulose, and lignin. Cellulose is the predominant polymer in lignocellulosic biomass and it consists of long homopolymer of $\beta(1\rightarrow4)$ linked D-glucose units. The strong beta acetal linkage enhances the ordering of glucose chain into a tightly packed crystalline structure resistant to hydrolysis. This characteristic differentiates cellulose from starch. Cellulose molecules are arranged into thin hair-like strands called microfibrils. These microfibrils are arranged in a mesh-like pattern along with hemicellulose and lignin, which link them together and help strengthen the plant cell wall (Klemm et al. 2005). Hemicellulose, the second most common component of lignocellulosic biomass, consists of several heterogeneous polymers such as xylan, arabinoxylan, glucuronoxylan, glucomannan, galactomannan, and glucoxylan, and is embedded in the cell walls of plants. Lignin, the non-carbohydrate component of lignocellulosic biomass and the third most common polymer found in plant biomass, is a heterogeneous polymer of three alcohol monomers, or monolignols: *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. Lignin provides strength and resistance against microbial diseases and pests to plants. The crystallinity of cellulose, cellulose sheathing by hemicellulose, protection of cellulose and hemicellulose by lignin, and the heterogeneous nature of biomass particles contribute to the recalcitrance of lignocellulosic biomass to hydrolysis (Figure 3.1; Chang and Holtzapple 2000; Mosier et al. 2005). A summary of composition of different agricultural residues and energy crops is shown in Table 3.1.

On the one hand, hydrolysis of cellulose component of lignocellulosic biomass requires cellulase, a group of enzymes (endoglucanase, exoglucanase, and β -glucosidase) that acting synergistically together, hydrolyze cellulose. On the other hand, complete hydrolysis of hemicellulose requires xylanase, β -xylosidase, and several other complimentary enzymes, such as acetylxyylan esterase, α -arabinofuranosidase, α -glucuronidase, α -galactosidase, ferulic and/or *p*-coumaric acid esterase (Figure 3.2a; Ezeji et al. 2007a). Activities of these enzymes, in addition to activities of cellulases on the cellulose component of biomass, result in the generation of a complex mixture of monomeric sugars such as glucose, galactose, xylose, arabinose, and acids (e.g., ferulic, *p*-coumaric, acetic, glucuronic) in biomass hydrolysates (Ezeji et al. 2007a). Acetic, ferulic, coumaric, and glucuronic acid inhibit the growth and ABE production by solventogenic *Clostridium* species (Ezeji et al. 2007b). For complete depolymerization of lignocellulosic biomass, therefore, it is difficult to totally avoid the generation of inhibitory compounds irrespective of the pretreatment and hydrolysis method used (Ezeji et al. 2007a).

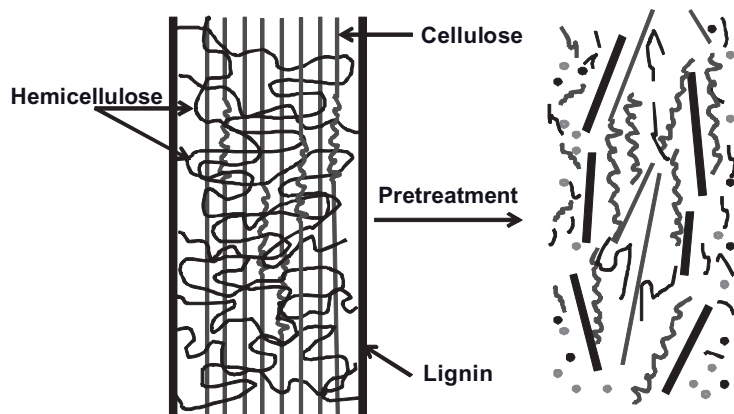


Figure 3.1. Schematic diagram depicting goals of pretreatment on lignocellulosic substrate. Reproduced from Mosier et al. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* 96:673–686. With permission.

Table 3.1. Composition of common agricultural residues and energy crops.

Agricultural Residues and Energy Crops	Cellulose (%)	Hemicellulose (%)	Lignin	References
Bagasse	44.3	26.0	18.9	Kelley et al. (2004)
Rice straw	47.0	26.0	17.0	Moniruzzaman (1996)
Wheat straw	39.8	27.3	22.6	Kristensen et al. (2008)
Corn stover	42.5	30.7	19.3	Ezeji (2008)
Corn cobs	45	35	15	Reshamwala et al. (1995)
Corn fiber	13.0	38.8	7.5	Noureddini et al. (2009)
DDGS	17.0	25.8	8.7	Noureddini et al. (2009)
Hardwood	40–55	24–40	18–25	Reshamwala et al. (1995)
Softwood	45–50	25–35	25–35	Reshamwala et al. (1995)
Swine waste	6.0	28	NA	Boopathy (1998)
Switch grass	45	31.4	12.0	Reshamwala et al. (1995)
Rye straw	33.1	22.2	19.8	Sun and Cheng (2005)
Bermudagrass	32.4	24.8	20.33	Sun and Cheng (2005)
Office paper	68.6	12.4	11.3	Wiseloge et al. (1996)
Poplar	49.9	17.4	18.1	Wiseloge et al. (1996)

DDGS, dried distillers' grains and solubles.

Pretreatment of Lignocellulosic Biomass and Generation of Microbial Inhibitors

One of the key steps in the conversion of lignocellulosic biomass to fermentable sugars is pretreatment. The goal of pretreatment is to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic chemical composition so that enzymatic hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved with greater yield (Figure 3.1; Mosier et al. 2005). Pretreatment of lignocellulosic biomass

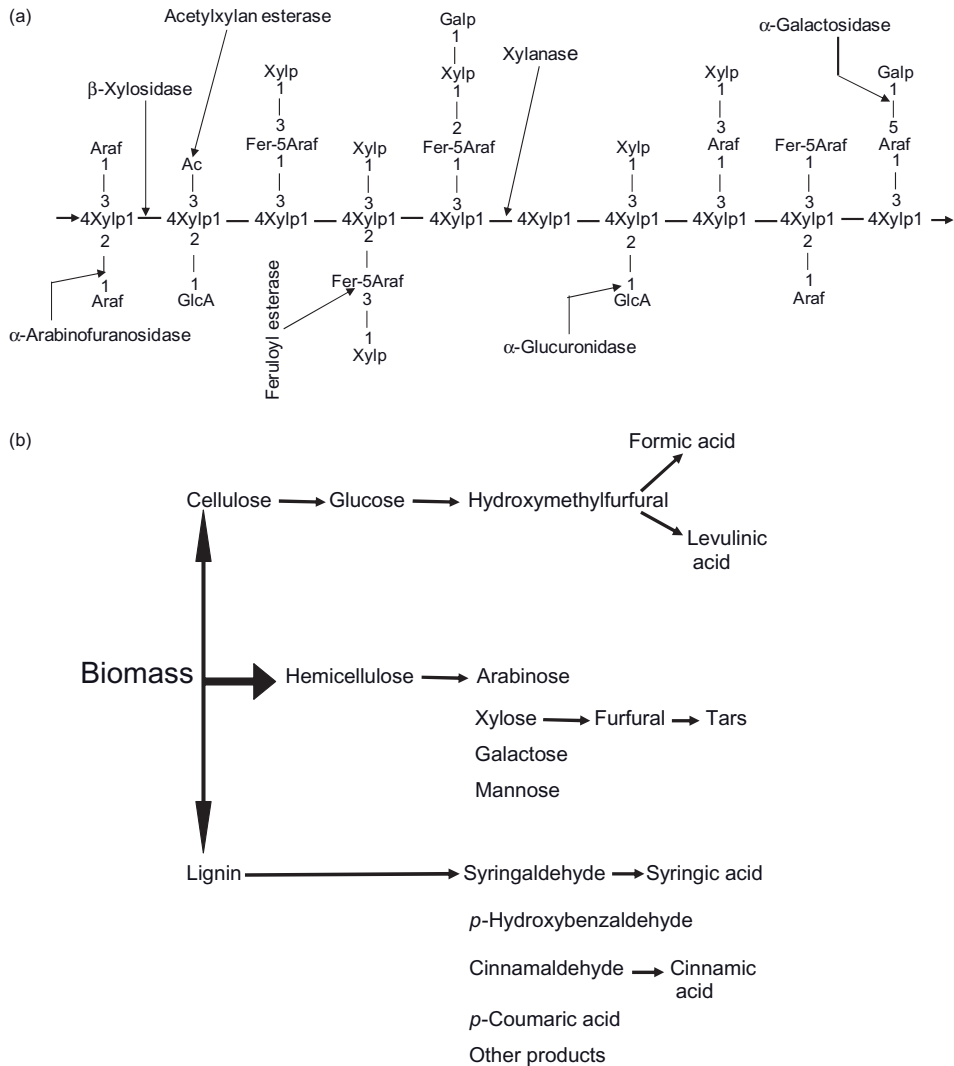


Figure 3.2. (a) Schematic of the basic structure of corn fiber heteroxylan and enzymes needed to degrade the polymer to monomeric compounds. Araf: arabinofuranose, Xylp: xylopyranose, Galp: galactopyranose, GlcA: glucuronic acid, Fer: ferulic acid, AC: acetylxylosterase. Reproduced from Ezeji et al. (2007). Bioproduction of butanol from biomass: From genes to bioreactors. *Curr. Opin. Biotechnol.* 18:220–227. With permission. (b) Composition of lignocellulosic biomass and potential degradation products. Schematic shows sequence of the conversion of biomass polymers to monomeric sugars and degradation products.

could also be conducted to disrupt and separate lignin from the hemicellulose component of the lignocellulosic biomass. For pretreatment to be effective, it must meet the following criteria: (1) simple to operate and inexpensive; (2) has the ability to expose the cellulose component of biomass and increase its vulnerability to enzymatic attack;

(3) does not degrade hydrolyzed sugars; (4) generates little or no microbial inhibitory products; and (5) must be environmentally compatible. Unfortunately, during pretreatment and hydrolysis of lignocellulosic biomass, degradation and hydrolysis products such as furfural, HMF, syringaldehyde, glucuronic acid, *p*-coumaric acid, ferulic acid, syringic acid, levulinic acid, and other phenolic compounds may be generated (Figure 3.2b; Martinez et al. 2001; Ezeji et al. 2007a,b). These compounds can inhibit growth of microbes including fermenting microorganisms (Martinez et al. 2001; Ezeji et al. 2007a,b; Ezeji and Blaschek 2008a). During an investigation on the effect of some of the lignocellulosic hydrolysates inhibitors on growth and ABE production by *Clostridium beijerinckii* 592, ferulic, and *p*-coumaric acids were found to be potent inhibitors of growth and ABE production (Figures 3.3 and 3.4). Interestingly, glucuronic acid and HMF were not inhibitory to the *C. beijerinckii* 592, but rather were stimulatory to growth and ABE production at concentrations up to 2.0 g/L (Figures 3.3 and 3.4; Ezeji et al. 2007b; Ezeji and Blaschek 2008a). Similar results were obtained when *C. beijerinckii* BA101 (Ezeji et al. 2007b) and other solventogenic *Clostridium* species (Ezeji and Blaschek 2008a) were used.

It is important to note the number and amount of inhibitors likely to be generated during pretreatment of biomass may depend on the type and intensity of pretreatment applied. Some of the processes currently used in the pretreatment of lignocellulosic biomass are subsequently described in this chapter.

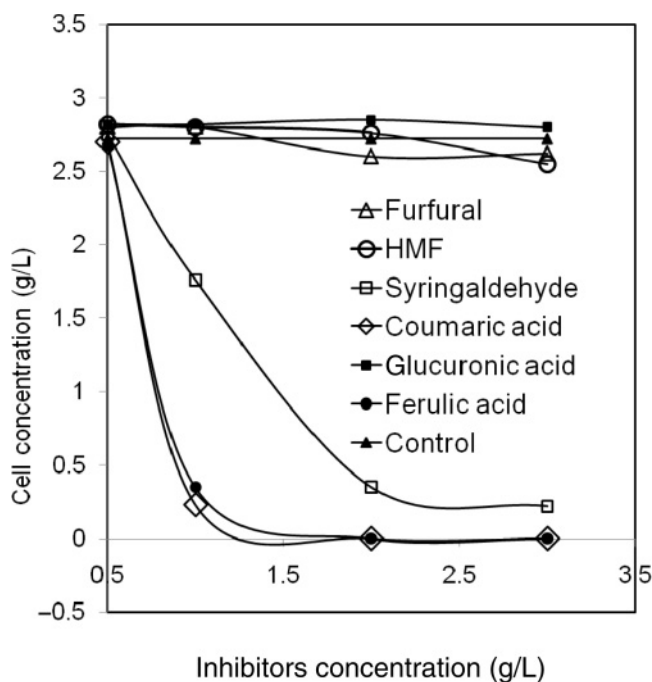


Figure 3.3. Effect of representative lignocellulosic biomass degradation products on the cell growth of *Clostridium beijerinckii* 592.

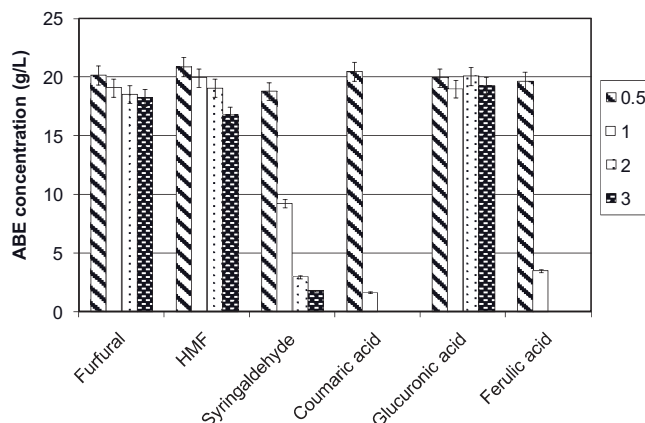


Figure 3.4. Effect of representative lignocellulosic biomass degradation products on ABE production by *Clostridium beijerinckii* 592.

Size Reduction

Size reduction is a mechanical pretreatment of lignocellulosic biomass with the objective being reduction of particle size of biomass by a combination of chipping and milling. During size reduction operation, lignocellulosic biomass material such as corn stover is reduced to 5 to 20 mm in size in a straw chopping machine. Following the chopping process is milling, which reduces the particle size further to <0.5 to 2.0 mm diameters using a hammer, ball, or pin mill. The reduction in particle size results to increase in biomass density and improves handling during subsequent pretreatments. More importantly, it leads to an increase of surface area of lignocellulosic biomass, which improves the contact of polymers and enzymes and ultimately, hydrolysis of biomass to fermentable sugars. A particle size reduction below 40 mesh (0.42 mm) has little or no effect on the hydrolysis of or sugar yield from lignocellulosic biomass (Chang and Holtzapple 2000).

Liquid Hot Water (LHW)

Noting that water goes into a gaseous phase at 100°C, hot water pretreatment is a process in which pressure is used to maintain water in a liquid state at high temperatures (140–230°C) as water comes into contact with biomass. The major objective of hot water pretreatment is to wet and solubilize lignocellulosic biomass components especially hemicellulose and make the cellulose component accessible to hydrolytic enzymes. About 40%–60% of the total biomass is solubilized during hot water pretreatment, with 4%–22% of the cellulose, 35%–60% of lignin, and all of the hemicellulose being removed (Mosier et al. 2005). LHW pretreatment enhances the cleavage of *o*-acetyl and uronic acid from hemicellulose to generate acetic, ferulic, glucuronic, and other organic acids, which decreases the pH of lignocellulosic biomass hydrolysates. The structural and chemical changes that occur in lignocellulosic biomass during hot water pretreatment are aided by the presence of these acids in solution. These acids catalyze the formation of monomeric sugars from xylan, arabinan, galactan, mannan polymers as well as aldehydes such as furfural from xylose and 5-HMF from glucose (Ezeji et al. 2007b).

Steam Pretreatment and Steam Explosion

Steam pretreatment is a process in which lignocellulosic biomass is treated with high-pressure (0.69–4.83 MPa) saturated steam (160–260°C) for several seconds to a few minutes (Sun and Cheng 2002). The pressure may be swiftly reduced so the material undergoes an explosive decompression (Sun and Cheng 2002). The only difference between steam pretreatment and explosion is the quick depressurization and cooling of the biomass after steaming at a predetermined length of time. Steam pretreatment or explosion helps to disrupt the integrity of lignin sheath (Figure 3.1) and solubilize the hemicellulose component to enhance accessibility of cellulose to enzymatic hydrolysis. Grous et al. (1986) pretreated chopped poplar with steam explosion, and subsequent enzymatic hydrolysis resulted in greater than 90% efficiency in 24 hours, compared with 15% hydrolysis of untreated poplar. Residence time, biomass particle size, temperature, and moisture content affect the efficiency of steam pretreatment and explosion (Duff and Murray 1996).

Acid Hydrolysis

Acid pretreatment can be conducted with concentrated or dilute mineral acids. Because concentrated acid is very corrosive, hazardous, and requires expensive reactors that are resistant to corrosion before it can be used, dilute acid is usually preferred for biomass pretreatment. Typically in dilute acid hydrolysis, biomass is soaked in a 1.0 wt% solution of sulfuric acid at a 10 wt% solids concentration for 24 hours. Excess liquid was removed by filtration, leaving particles of a 20%–30% solids concentration. The solids are loaded in a reactor heated at 120–160°C for a predetermined length of time. The main reaction that occurs during dilute acid pretreatment is the disruption of lignocellulosic structure and the hydrolysis of hemicellulose, especially arabinoxylan. The solubilization of hemicellulose and lignin by acid treatment exposes the cellulose component for enzymatic hydrolysis. A dilute acid pretreatment process is typically conducted in two primary treatment regimens: high temperature short time (HTST) or low temperature long time (LTLT). Dilute acid pretreatment processes have been used to pretreat corn fiber and dried distillers' grains and solubles (DDGS), which upon enzymatic hydrolysis was used to conduct ABE fermentation using various solventogenic *Clostridium* spp. (Qureshi et al. 2007; Ezeji and Blaschek 2008a). Dilute acid pretreated corn fiber (8.4% total solid loading) generated 54.3 g/L total sugars upon enzymatic hydrolysis (Qureshi et al. 2007), while DDGS (15% total solid loading) generated 52.6 g/L total sugars upon enzymatic hydrolysis (Ezeji and Blaschek 2008a).

Alkaline Hydrolysis

Alkali pretreatment is a process in which alkaline solutions like NaOH or KOH are used to remove and solubilize lignin component of lignocellulosic biomass and efficiently increase the accessibility of enzyme to the cellulose component. Using alkali to treat lignocellulosic biomass has been known for years to improve cellulose digestibility. For example, lime has been used to pretreat poplar wood (150°C for 6 hours with 14-atm oxygen; Chang et al. 2001), switchgrass (100°C for 2 hours; Chang et al. 1997), wheat straw (85°C for 3 hours; Chang et al. 1998), corn stover (100°C for 13 hours; Karr and Holtzapple 1998, 2000), and sugarcane bagasse (ambient conditions up to 192 hours; Playne 1984). During dilute NaOH pretreatment of lignocellulosic biomass, the mechanisms of reaction are by solvation and saponification (Hendriks and Zeeman 2009). The intermolecular ester bonds cross-linking

xylan hemicellulose, other hemicellulose, and lignin (Figure 3.2a) are saponified. During solvation and saponification reactions, the lignocellulosic material is swollen, leading to an increase in surface area, decrease in crystallinity of material, disruption of lignin structure, and separation of structural linkages between lignin and carbohydrates (Fan et al. 1987; Sun and Cheng 2002). Alkaline pretreatment of chopped rice straw with 2% NaOH and 20% solid loading at 85°C for 1 hour decreased the lignin by 36% and substantially increased enzymatic cellulose hydrolysis (Zhang and Cai 2008).

Ammonia Fiber Expansion (AFEX) Pretreatment

AFEX pretreatment (Dale 1986) is an alkaline pretreatment process that alters physicochemical structure of lignocellulosic biomass at moderate temperatures (60–100°C) and high pressures (250–300 psi). Like the alkali pretreatment, AFEX pretreatment results in increased wetting of the treated biomass (Sulbaran de Ferrer et al. 1997; Shishir et al. 2007), decrystallization of cellulose (Gollapalli et al. 2002), partial depolymerization of hemicellulose, cleavage of lignin–carbohydrate complex (LCC) linkages (Shishir et al. 2007), and increase in surface area due to structural disruption.

In a typical AFEX pretreatment process using corn stover (Shishir et al. 2007), corn stover is adjusted to 60% moisture (kilogram water/kilogram dry biomass) before being transferred to a high-pressure Parr reactor, and liquid ammonia (1 kg of ammonia/kg of dry biomass) is slowly added to the reactor. The temperature is raised and maintained at 90°C for a period of 5 minutes. Following a 5-minute residence time, the pressure is quickly released. The sudden drop in reactor pressure results in fast vaporization of ammonia, causing an explosive decompression and disruption of the biomass. The pretreated corn stover is stored under a fume hood overnight, during which the residual ammonia is vaporized. Subsequently, the AFEX pretreated corn stover is either subjected to enzymatic hydrolysis or stored in a freezer until further use.

The AFEX pretreatment process has some unique characteristics that distinguish it from other pretreatment methods and they are as follows (Teymouri et al. 2005): (1) a significant amount of the ammonia used in the pretreatment is recovered and reused; (2) AFEX is basically a dry to dry process because there is no waste or liquid stream from the process; (3) pretreated biomass is stable for long periods and can be used with great solid loadings in enzymatic hydrolysis and fermentation processes; (4) AFEX process yields intact cellulose and hemicellulose polymers with little or no degradation; (5) no need for neutralization prior to the enzymatic hydrolysis of AFEX-pretreated biomass. The AFEX pretreatment process was used recently to pretreat DDGS, and subsequently fermented into ABE (Ezeji and Blaschek 2008a). In this investigation, various *Clostridium* species were tested for the ability to ferment AFEX-pretreated DDGS to ABE. The AFEX pretreated DDGS (15% total solids loading) generated 41.4 g/L total sugars upon enzymatic hydrolysis comparable to that generated by hot water pretreated samples.

Electrolyzed Water Pretreatment

Electrolyzed water is a technique first developed in Japan in the 1990s. The concept involves electrolysis of water containing a small amount of sodium chloride (0.1%) in an electrolysis chamber where anode and cathode are separated by a bipolar membrane imparting unique characteristics to the water collected from the two electrodes. The water from the anode normally has a pH of ≤ 2.7 and an oxidation reduction potential (ORP) of $>1,100$ mV. The

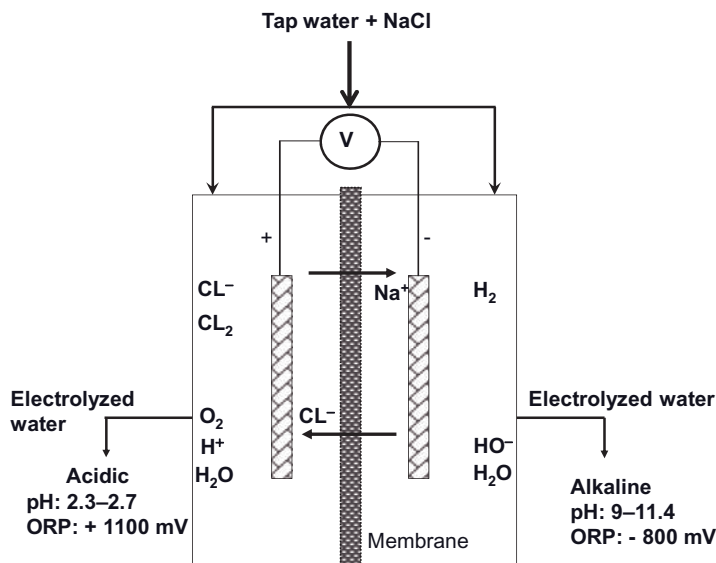


Figure 3.5. Schematic diagram depicting electrolyzed water generation unit. Electrolyzed water is produced by the electrolysis of tap water containing dissolved NaCl (0.1%, w/w).

water produced from the cathode side has a pH of >11.4 and ORP of <−795 mV. The schematic diagram depicting the process is included in Figure 3.5. The low pH acidic electrolyzed water (AEW) and the high pH alkaline electrolyzed water have been used recently to pretreat DDGS, and subsequently for fermentation into ABE (Wang et al. 2009a). Scanning electron micrograph (SEM) images of DDGS pretreated with AEW showed the crystalline structure of the DDGS was disrupted by the pretreatments with AEW at pH 2.7 (Wang et al. 2009a). A major advantage of using electrolyzed water is that it is produced using water with no added chemicals other than very dilute sodium chloride.

Treatment of Lignocellulosic Hydrolysates for Inhibitor Removals

A major problem associated with the fermentation of hydrolysates of lignocellulosic biomass is the presence of a broad range of degradation and products (nonsugar) of hemicellulose hydrolysis that inhibit fermenting microorganisms (Ezeji et al. 2007a,b). These inhibitors can be divided into three groups (Figure 3.2a,b) on the basis of origin: compounds released from the hemicellulose component of lignocellulosic biomass (e.g., acetic, ferulic, glucuronic acid); lignin degradation products (e.g., syringaldehyde, syringic acid, and phenolic compounds); and sugar degradation products (e.g., furfural, HMF, formic, and levulinic acid; Ezeji et al. 2007a,b). The type, number, and concentrations of these inhibitory compounds vary depending on the type of lignocellulosic biomass and pretreatment conditions. For efficient utilization of lignocellulosic hydrolysates by fermenting microorganisms, hydrolysates must be detoxified to rid or reduce concentrations of these inhibitors to tolerant amounts prior to fermentation. Villarreal et al. in 2006 evaluated five different detoxification procedures

to remove microbial inhibitors from eucalyptus hemicellulose hydrolysates prior to xylitol production by *Candida guilliermondii*. The detoxification methods employed in the investigation include treatment with activated charcoal and four different resins (cationic and anionic) connected in sequence. Among the detoxification methods employed, ion exchange resins were more efficient in the removal of all three major groups of inhibitory compounds without sugar loss than activated charcoal (Villarreal et al. 2006). Marchal et al. (1986) found hydrolysates obtained by enzymatic saccharification of wheat straw or corn stover pretreated by steam explosion in acidic conditions could not be fermented into ABE. A simple treatment involving heating the hydrolysates in the presence of calcium or magnesium compounds such as $\text{Ca}(\text{OH})_2$ or MgCO_3 at neutral pH values restored normal fermentation of these hydrolysates to ABE. Qureshi et al. (2008a), during evaluation for use of corn fiber as a substrate for ABE production by *C. beijerinckii* BA101, reported a maximum cell concentration of 0.65 g/L when dilute acid pretreated corn fiber hydrolysates (54.3 g/L total sugar) were the substrate compared to a maximum cell concentration of 3.37 g/L obtained from the control (pure mixed sugars). *C. beijerinckii* BA101 grown in dilute acid pretreated corn fiber hydrolysates experienced a longer lag phase than the control, and the culture was unable to make a transition from the acidogenic to solventogenic ABE production phase, consistent with it being inhibited by lignocellulosic degradation products. When dilute acid pretreated corn fiber hydrolysates were treated with XAD-4 resin prior to fermentation, growth and ABE production by *C. beijerinckii* BA101 increased by approximately 300% and 500%, respectively. Soni et al. (1982), in addition, reported that bagasse and rice straw hydrolysates were inhibitory to *Clostridium saccharoperbutylacetonicum*.

A fungus, *Coniochaeta ligniaria* NRRL30616, which metabolizes furfural, 5-HMF, aldehydes, aromatic, and aliphatic acids, was recently isolated (Nichols et al. 2008). The investigators found that *C. ligniaria* NRRL30616 grew in corn stover dilute-acid hydrolysates, and compounds representing all of the three groups (aromatic and aliphatic acids, aldehydes, and phenolic compounds) of inhibitory products were removed during the course of fungal growth. Fungal laccase and peroxidase enzymes, in addition, have been used to detoxify wood hydrolysates (Jönsson et al. 1998; Martin et al. 2002). Furthermore, Larsson et al. (2001) expressed laccase in *Saccharomyces cerevisiae* and the mutant had increased resistance to phenolic compounds. These developments show that biological inhibitor abatement for reducing or eliminating inhibitory compounds from biomass hydrolysates appears to be a promising method for detoxification but drawbacks in terms of cost-effectiveness and competition for substrate could be a problem. The development of fermenting strains that can tolerate greater concentrations of inhibitory compounds generated during acid pretreatment and hydrolysis of lignocellulosic biomass remains a priority.

Butanol Production from Agricultural Residues

Traditionally, the primary substrates for the industrial production of acetone–butanol were corn and molasses. The use of molasses as a fermentation substrate offers some advantages as compared to corn, such as easy handling and utilization by saccharolytic *Clostridium* species. Cane molasses was used in the commercial production of butanol in South Africa until about 1980 (Ezeji et al. 2004a). Solventogenic *Clostridium* species express the genes for potent amylolytic enzymes constitutively and do not need amylases added to substrate to metabolize starchy products. For this reason, other starchy substrates such as millet, wheat,

rice, cassava, tapioca, and potatoes have been used successfully for growth and ABE production by solventogenic *Clostridium* species. The United States has the capacity to produce 13 billion gallons of biofuel per year from corn alone, and any further increase in biofuel production will most likely come from utilization of feedstocks other than corn grain because of limitations in supply (Gray et al. 2006). Production of butanol from low-cost lignocellulosic biomass that does not compete with food crops may be the important aspect to meeting the DOE target (to blend 7.5 billion gallons of renewable fuels into gasoline by 2012), and for biobutanol production to become economically viable as well as sustainable (Ezeji and Blaschek 2008b).

Substrate cost has long been recognized as having the most influence on butanol price and has been identified as a major factor affecting economic viability of butanol production by fermentation (Qureshi and Blaschek 2000). To produce butanol at a competitive price, the use of more economic agricultural residues such as DDGS, corn fiber, corn stover, wheat straw, rice straw, and wood has been evaluated by many investigators including the laboratories of the authors. Butanol-producing cultures are able to use a wide variety of carbohydrates such as starch, cellobiose, sucrose, glucose, fructose, mannose, dextrin, galactose, xylose, and arabinose. The use of these carbohydrates by butanol-producing cultures illustrates the potential to ferment sugars derived from hydrolysates of agricultural residues to butanol (Qureshi and Ezeji 2008).

Dried Distillers' Grains and Solubles (DDGS)

Dry and wet mill ethanol production from corn starch is regarded as an essentially mature technology for producing bioethanol. Currently, dry-grind ethanol plants produce the majority of ethanol fuel (ca. 60%) in the United States. With concerns regarding net energy balance and food compared to fuel debate, ethanol production from corn is expected to stabilize (von Braun 2007). Some incremental increases in energy efficiency of this process, however, can be expected as coproduct (DDGS) utilization is incorporated into next-generation plants. Currently, DDGS from corn ethanol production is used as animal feed. Seven million metric tons of DDGS were estimated to have been produced from corn ethanol processing in the United States at the end of 2008 (Blaschek et al. 2010). DDGS conversion into liquid fuels and incorporation of DDGS into the ethanol biorefinery industry will increase the efficiency and profitability of corn ethanol plants (Blaschek et al. 2010).

Ezeji and Blaschek (2008a) conducted a comprehensive study evaluating fermentation capability of dilute acid, LHW, or AFEX pretreated DDGS hydrolysates by solventogenic *Clostridium* species. The ABE production profiles over the course of a 120-hour fermentation of detoxified dilute acid pretreated DDGS hydrolysates elucidated that *C. beijerinckii* 260, *Clostridium acetobutylicum* 824, *Clostridium saccharobutylicum* 262, *C. beijerinckii* 592, and *C. beijerinckii* BA101 produced maximum ABE concentrations of 8.1, 4.9, 12.1, 7.5, and 6.8 g/L, respectively, and total residual acids of 4.7, 4.2, 5.2, 4.0, and 4.1 g/L, respectively (Table 3.2). During the 72-hour fermentation of LHW and AFEX pretreated DDGS and contained 48.8 and 41.4 g/L total sugars, *C. beijerinckii* 260, *C. acetobutylicum* 824, *C. saccharobutylicum* 262, *C. beijerinckii* 592, and *C. beijerinckii* BA101 produced maximum ABE concentrations of 12.8, 11.4, 10.5, 12.9, and 11.5 g/L, respectively, and total residual acids of 4.0, 5.3, 4.9, 7.4, and 4.4 g/L, respectively. For AFEX pretreated DDGS medium, *C. beijerinckii* 260, *C. acetobutylicum* 824, *C. saccharobutylicum* 262, *C. beijerinckii* 592, and *C. beijerinckii* BA101 produced maximum ABE concentrations of 10.2, 9.0, 7.9, 11.6, and 10.4 g/L, respectively, and total residual acids of 5.4, 4.0, 5.2, 4.3, and 5.1 g/L. Importantly, LHW

Table 3.2. Production of butanol from lignocellulosic biomass. Table shows type of pretreatment and biomass detoxification processes employed prior to ABE fermentation.

Substrate	Hydrolysis Method	Treatment to Remove Inhibitors	Culture	Butanol Produced (g/L)	ABE Produced (g/L)	Yield (g ABE/g sugar)	References
Bagasse	Alkali pretreated + enzyme hydrolyzed	Ammonium sulfate + activated carbon	<i>Clostridium saccharoperbutylacetonicum</i> ATCC 27022	11.0	14.0	0.30	Soni et al. (1982)
Rice straw	Above	Above	Above	10.0	13.0	0.28	Soni et al. (1982)
Wheat straw	Alkali pretreated + enzyme hydrolyzed	None	<i>Clostridium acetobutylicum</i> IFP 921	10.3	17.7	0.22	Marchal et al. (1984)
Corn stover	Steam explosion	Ca(OH) ₂	<i>C. acetobutylicum</i> NCIB644	8.0	12.8	0.27	Marchal et al. (1986)
Corn fiber	Dilute sulfuric acid	XAD-4 resin	<i>Clostridium beijerinckii</i> BA101	6.4	9.3	0.39	Qureshi et al. (2008a)
DDGS	AFEX	None	<i>C. beijerinckii</i> 260	7.7	10.2	0.32	Ezeji & Blaschek (2008a)
Above	Hot water	None	<i>C. acetobutylicum</i> 824	8.5	11.4	0.31	Ezeji & Blaschek (2008a)
Above	Dilute sulfuric acid	Over-liming	<i>Clostridium saccharobutylicum</i> 262	7.2	12.1	0.35	Ezeji & Blaschek (2008a)
			<i>C. beijerinckii</i> 260	5.6	8.1	0.31	Above
			<i>C. beijerinckii</i> 592	5.2	7.5	0.30	Above
			<i>C. acetobutylicum</i> 824	3.8	4.9	0.31	Above

ABE, acetone butanol ethanol; DDGS, dried distillers' grains and solubles; AFEX, ammonia fiber expansion.

and AFEX pretreated DDGS hydrolysates were not subjected to any detoxification process prior to fermentation and there was no significant difference between total ABE produced from DDGS and that of the control (mixed glucose-mannose-arabinose-xylose [GMAX]) with corresponding amounts of GMAX. Wang et al. (2009a), in addition, investigated the effect of total solids loading on pretreatment of DDGS by electrolyzed water and ABE fermentation. DDGS samples pretreated at 30% solids loading with alkaline electrolyzed water (ALEW) produced 16.9 g/L total ABE in 72 hours upon fermentation by *C. beijerinckii* P260.

To improve *C. beijerinckii* BA 101 tolerance to toxic compound generated during pretreatment and hydrolysis of lignocellulosic biomass and enhance fermentation capability of AEW-pretreated DDGS, Wang et al. (2009b) conducted microorganism adaptation studies where *C. beijerinckii* BA 101 was treated with increasing amounts of acid-pretreated DDGS hydrolysates containing lignocellulosic degradation compounds that are inhibitory to solventogenic *Clostridium* species. Hydrolysates-adapted *C. beijerinckii* BA101 strains were able to adjust to the inhibitory environment in less than 20 hours and produce approximately the same amount of ABE at a comparable time to that of the control fermentation.

Corn Fiber and Corn Stover

Corn fiber is a coproduct of the corn wet milling industry. It is a mixture of corn kernel hulls and residual starch not extracted during the wet milling process. Corn fiber is composed of approximately 40% hemicellulose, 12% cellulose, 25% starch, 10% protein, 3% oil, and 10% other substances such as ash and lignin (Singh et al. 2003). Approximately 6.3×10^6 dry tons of corn fiber is produced annually in the United States. Typically 4.5 lb of corn fiber is obtained from a bushel (56 lb) of corn, which can be converted to about 3.0 lb of fermentable sugars (Ezeji et al. 2006). The major fermentable sugars from hydrolysis of corn fiber are glucose, xylose, and arabinose (Ezeji and Blaschek 2008a; Nouredini and Byun 2010). Small amounts of mannose (Ezeji and Blaschek 2008a) and galactose (Nouredini and Byun 2010) have also been measured in corn fiber hydrolysates.

Economically, it is important that these mixed sugars present in corn fiber hydrolysates be fermented to butanol for this renewable biomass to be used as feedstock for butanol production. Solventogenic *Clostridium* species have an added advantage over many other cultures as they can utilize both hexose and pentose sugars (Ezeji et al. 2007a,b; Ezeji and Blaschek 2008a) released from lignocellulosic biomass upon hydrolysis to produce butanol. *C. beijerinckii* BA101 effectively ferments detoxified dilute acid pretreated corn hydrolysates to produce butanol (Table 3.2; Qureshi et al. 2008a). Similarly, Parekh et al. (1988) produced butanol from hydrolysates of corn stover using *C. acetobutylicum* P262. Marchal et al. (1986), in addition, fermented acid pretreated corn stover hydrolysates to produce 12.8 g/L acetone–butanol using *C. acetobutylicum* NCIB644 (Table 3.2). In another development, Zhu et al. (2002) produced butyric acid, an intermediate ABE fermentation product, from acid hydrolysates of corn fiber by *Clostridium tyrobutyricum* in a fibrous-bed bioreactor. This fermentation process can improve butanol yield, reduce butanol production costs, and improve the economics of butanol fermentation. Importantly, butyric acid can easily be incorporated into fermentation medium and converted to butanol during ABE fermentation (Tashiro et al. 2004).

Wheat Straw

About 77.1 million metric tons of wheat straw is produced in the United States annually, and this quantity has the potential to produce 54 million metric tons of fermentable sugars (<http://>

www.biofuelscenter.org). Wheat straw, which is composed of 67% of cellulose and hemicellulose (Table 3.1), can serve as a cheap substrate for butanol production by solventogenic *Clostridium* species. Qureshi et al. (2007, 2008b,c) have pioneered research on potential use of wheat straw as substrate for ABE production. During one investigation, five different processes were evaluated for ABE from wheat straw by *Clostridium beijerinckii* P260 (Qureshi et al. 2008b). The five processes were fermentation of pretreated wheat straw (Process I), separate hydrolysis and fermentation of wheat straw to ABE without removing sediments (Process II), simultaneous hydrolysis and fermentation of wheat straw without agitation (Process III), simultaneous hydrolysis and fermentation with additional sugar supplementation (Process IV), and simultaneous hydrolysis and fermentation with butanol recovery by gas stripping (Process V). In these studies, process IV and V achieved maximum ABE concentrations of 17.92 g/L and 22.42 g/L, resulting in ABE productivities of 0.19 and 0.31 g/L/h, respectively. In the control experiment (glucose), an ABE productivity of 0.30 g/L/h was achieved (Qureshi et al. 2008b).

To improve *C. beijerinckii* 260 tolerance to toxic compounds generated during pretreatment and hydrolysis of wheat straw biomass and enhance fermentability of acid pretreated wheat straw, Qureshi et al. (2008d) used alkaline peroxide and electrodialysis treatment methods to detoxify acid pretreated wheat straw hydrolysates prior to ABE fermentation. A maximum ABE concentration of 22.17 g/L with reactor productivity of 0.55 g/L/h was produced, compared to ABE concentration of 21.37 g/L with reactor productivity of 0.30 g/L/h produced by the control (glucose). To improve ABE productivity further, Qureshi et al. (2008c) used *C. beijerinckii* P260 to produce butanol from wheat straw hydrolysates in a fed-batch reactor with simultaneous ABE recovery by gas stripping. A total of 192.0 g ABE was produced in a 2.5 L bioreactor (1 L reaction volume) at the end of fermentation, resulting in an ABE productivity of 0.77 g/L/h and ABE yield of 0.44. Simultaneous hydrolysis and fermentation of wheat straw to butanol and/or fed-batch fermentation of wheat straw hydrolysates and simultaneous butanol recovery by gas stripping are, therefore, attractive options with great potential of replacing glucose for butanol production.

Conclusion

With continuous world population growth and energy demand, current production of liquid biofuel (ethanol) from food crops such as corn and sugar cane is unsustainable. The use of agricultural residues to produce liquid biofuels—in particular, the production of butanol—holds great interest as a means for generating sustainable transportation fuel and feedstock chemicals. The possibility of using agricultural residues such as corn fiber, corn stover, wheat straw, as well as energy crops such as switchgrass, *Miscanthus*, and so on for butanol production and incorporating gas stripping in an in-line product recovery process has generated considerable interest. Simultaneous butanol fermentation and recovery has dramatically improved production of butanol from wheat straw. Considerable progress has been made in the development of biomass pretreatment and hydrolysis technologies. Progress has also been made on the improvement of some technologies that were previously developed so that use is more environmentally compatible and generating little or no lignocellulosic degradation compounds that are toxic to fermenting microorganisms. Availability of efficient biomass pretreatment technology will help in utilizing bio-based conversion of agricultural residues into value-added products to become attractive. By employing in-line recovery systems during butanol fermentation, substrate inhibition and butanol toxicity to the culture

are drastically reduced, with ABE productivity dramatically increased. Given that butanol is an excellent potential fuel and the United States is rich in lignocellulosic biomass, butanol production from agricultural residues is crucial in future energy production endeavors.

Acknowledgments

This work was supported by funding from Northeast Sungrant (Cornell University) Award/Contract # GRT00012344, National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2006-35504-17419, and Seed grant from Ohio Agricultural Research and Development Center (OARDC), Wooster.

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Chapter 4

Practical Aspects of Methane Production from Agricultural Wastes

Largus T. Angenent and Norman R. Scott

Abstract

Anaerobic digestion is a proven technology for bioconversion of agricultural wastes that are high in organic material. This engineered process with an undefined mixed microbial culture is based on the carboxylate platform (i.e., intermediates are channeled through short-chain fatty acids). The advantage of a mixed culture is that the waste material can be complex and variable in composition over the operating period, while sterilization is not necessary. The anaerobic food web is very efficient in transforming complex organic compounds into methane for stable digesters, because almost all intermediate products in the food chain are converted into methane with very low concentrations of carboxylates in the digester effluent and hydrogen in the off gas. The reason for such a high conversion is that the final products (methane and carbon dioxide) freely bubble out of the solution, which results in the circumvention of product inhibition and separation costs. However, methane formation is sensitive to instabilities. Thus, practical studies are still being performed to answer the following questions: How do mixed substrates, such as agricultural residues and manures, affect methane fermentation during co-digestion? How can performance and stability be improved? What are the limitations of methane fermentation? What are the economic and environmental benefits of conversion of agricultural residues to methane? What are the future directions for improved methane fermentation? In this chapter we will address these questions for anaerobic digestion of slurries and solid wastes.

Methanogenic Populations Drive the Anaerobic Food Web of Digesters

Methanogenic Food Web

Anaerobic digestion of readily degradable organic feedstock (biomass) is a very efficient energy recovery system because the final products—methane and carbon dioxide (CO₂)—are

automatically and constantly removed from the process by degassing (bubble formation), while cell yields for anaerobic microbial growth are low. This results in a relatively high level of energy recovery by the production of a reduced carbonaceous energy carrier—methane. No artificial fermentation product removal system is necessary, and under healthy digestion conditions all intermediate fermentation products are maintained at very low concentrations (i.e., no other product that lowers efficiency is formed; De Schamphelaire and Verstraete 2009). Under these conditions, the anaerobic food web, which consists of hydrolytic and fermentative bacteria, obligatory H_2 -producing acetogenic bacteria, H_2 -utilizing acetogenic or homoacetogenic bacteria, hydrogenotrophic methanogenic archaea, and acetoclastic methanogenic archaea, is balanced to maintain extremely low product concentrations at each trophic level, and in other words pulls all reactions to completion. This results in an optimum system to convert a complex and often nonsoluble substrate into methane via the intermediate fermentation products acetate and hydrogen. Because acetate, butyrate, and propionate are all intermediate products of this food web and are collectively referred to as carboxylates, anaerobic digestion is part of the carboxylate platform as an alternative to the better known sugar and thermochemical platforms of the biorefinery concept.

Acetate Oxidation

According to conventional knowledge, in this anaerobic food web ~30% of the methane is generated via hydrogen (with CO_2 as the electron acceptor) by hydrogenotrophic methanogens and ~70% is generated via acetate by acetoclastic methanogens. The latter process is referred to as acetate cleavage (Smith and Mah 1980), and occurs under close-to-optimum environmental conditions without reactor upsets (Mechanism I in Table 4.1). When the environmental conditions are not as favorable (e.g., stressed conditions), an alternative mechanism exists to convert acetate into methane, reducing the percentage of methane that is generated from acetate through acetate cleavage. This is referred to as acetate oxidation as part of a two-step process in which acetate is first oxidized to H_2 and CO_2 by homoacetogenic bacteria (in reverse) and H_2/CO_2 are then converted to CH_4 by hydrogenotrophic methanogens (Mechanism II in Table 4.1; Barker 1936; Zinder 1984; Zinder 1994).

The first step of bacterial acetate oxidation has a positive standard free energy (Mechanisms II[a] in Table 4.1), resulting in a thermodynamically unfavorable reaction at biological standard conditions. At homoacetogenic nonequilibrium cases with the presence of methanogens in the anaerobic food web, the reaction sum becomes thermodynamically favorable provided

Table 4.1. Free energy yield at physiological standard conditions ($T = 25^\circ C$, $pH = 7$, $P = 1$ atm).

Mechanism	$\Delta G^{\circ'}$ (kJ/mol)	Reference
I: $CH_3COO^- + H_2O_{(l)} \leftrightarrow CH_{4(g)} + HCO_3^-$	-31.0	(Thauer et al. 1977)
	-31.2	(Amend and Shock 2001)
II(a): $CH_3COO^- + 4H_2O_{(l)} \leftrightarrow 2HCO_3^- + 4H_{2(g)} + H^+$	+104.6	(Thauer et al. 1977)
	+104.2	(Amend and Shock 2001)
II(b): $4H_{2(g)} + HCO_3^- + H^+ \leftrightarrow CH_{4(g)} + 3H_2O_{(l)}$	-135.6	(Thauer et al. 1977)
	-135.4	(Amend and Shock 2001)
II Sum: $CH_3COO^- + H_2O_{(l)} \leftrightarrow CH_{4(g)} + HCO_3^-$	-31.0	(Thauer et al. 1977)
	-31.2	(Amend and Shock 2001)

that an extremely low hydrogen partial pressure is maintained by the second step (Mechanisms II[b] in Table 4.1). Therefore, the relationship between an acetate-oxidizing bacteria and a hydrogenotrophic methanogen is a syntrophic relationship. Even though thermodynamically Mechanism II becomes as favorable as Mechanism I, the overall energy yield has to be shared between the two syntrophic members. Syntrophic acetate oxidation associations have been observed in a variety of anaerobic systems (Petersen and Ahring 1991; Schnürer et al. 1994, 1999; Shigematsu et al. 2004; Schwarz et al. 2007). In such systems, acetate-oxidizing bacteria play an extremely important role guaranteeing the proper functioning of the anaerobic digestion by preventing acetate accumulation. This alternative pathway is, therefore, important to maintain stability in engineered systems.

Agricultural Wastes and Anaerobic Digestion

Animal Wastes on the Farm

Because animal wastes consist of high solid levels (e.g., 20–100 g volatile solids [VS]/L for swine waste [2%–10% VS]), anaerobic treatment of animal waste offers many advantages over aerobic treatment, such as low electricity requirements (no O₂ addition is required), greater than 50% solids reduction, value-added biogas production, nutrient conservation, and odor reduction. However, post-treatment of anaerobic effluent may be necessary to convert, remove, and possibly recover nutrients. The liquid fraction of the treated animal waste from the digester must be recovered and applied to agricultural lands and this may cause odor, pathogen distribution in the environment (Collick et al. 2006), nitrogen pollution of ground water (Kross et al. 1993), nitrogen and phosphorus run off (Gerard-Marchant et al. 2005), and dissipation of antibiotic-resistant genes into the environment (Chee-Sanford et al. 2001; Aminov et al. 2002; Angenent et al. 2008).

Despite the need for further treatment to prevent environmental problems, the increasing size of confined animal feeding operations (CAFOs), encroaching urbanization, greenhouse gas emissions, heightened awareness of local air pollution (including odors and ammonia), and contamination of water have created strong incentives to develop long-term environmentally sound manure management practices and systems that include anaerobic digesters. The process of anaerobic digestion is not new and has been utilized for decades to manage animal wastes. More recently the opportunity to capture methane to develop a combined heat and power (CHP) system has attracted much attention in developed countries. The heat and electricity generation on the farm (cogeneration) has led to increasing numbers of anaerobic digesters.

With the further development of net metering (i.e., selling to the electrical grid) for farm anaerobic digesters in many localities, the economic benefits of installing digesters are enhanced. However, researchers still look for other biogas utilization systems besides CHP because, from the perspective of simplicity of operation and thermodynamic efficiency, the most desirable and least expensive option is to use biogas in a boiler or other combustion process for heat generation. To benefit from this, however, a proximate demand for this heat must be available. An alternative option is to upgrade biogas (50%–60% methane) to pipeline natural gas quality (95%–98% methane). To upgrade biogas, a system that includes pressurization and removal of contaminants (particularly hydrogen sulfide) and CO₂ must be procured. Thus, this option is limited to large CAFOs, a community digester receiving wastes from numerous farms, or a gas pipeline connection fed by a number of farms.

The economic assessment of farm-based anaerobic digesters is complex because each farm digester system is specifically designed for that site. For example, the variations within New York State include farm systems that do not generate electricity, farms with microturbines for CHP, and farms with internal combustion engines. One study for the New York State Energy Research and Development Authority (NYSERDA) showed that the net predicted annual benefit per cow for five farms with digesters varied from $-\$106$ to $+\$299$ (Gooch et al. 2007). The negative or low benefit per cow was on farms with only dairy manure digestion while the farm showing a positive benefit was receiving food wastes (co-digestion), which increased biogas production and was paid a “tipping fee” to accept the wastes.

Co-Digestion

As stated above, a successful economic outcome for anaerobic digesters may be dependent on co-digestion of, for example, animal manures with other organic feedstocks (only when other feedstocks are available locally). In addition to crops and crop residues, the use of food and organic industrial wastes offers excellent possibilities to develop waste management opportunities for these wastes and to avoid increasingly costly and limited landfill disposal. Manure co-digestion increases the buffering capacity of substrate mixtures and adds nutrients that can increase methane yields and substrate affinities compared with digestion of one substrate (Mladenovska and Ahring 2000; Mladenovska et al. 2003). Despite the perceived benefits of co-digestion, the overall and specific knowledge of adding organic feedstocks relative to single substrates is very limited (both the synergistic and antagonistic effects). Therefore, we initiated a study to evaluate the potential of co-digestion of dairy manure with a variety of organic substrates. The results of ~175 individual biochemical methane potentials (BMPs) on more than 30 different substrates showed that substrates rich in lipids and/or carbohydrates with a high VS content are good candidates for co-digestion with dairy manure (Figure 4.1; Labatut and Scott 2008). A critical parameter to estimate the BMP of a myriad of substrates is the substrate biodegradability and we suggest that future research needs to develop models to estimate the methane potential of the numerous farm waste options based on biodegradability of substrates.

From a logistic and economic perspective, the availability of food wastes and distance from source to farm anaerobic digester is critical because of transportation costs and energy consumption. The development of geographical information systems (GIS) offers a useful way to connect food waste sources to existing digesters (Ma et al. 2005). We developed a web site (<http://wastetoenergy.bee.cornell.edu/>) to identify the location of dairy farms and sources of food waste from food processors, restaurants (including fast food), universities and colleges, K-12 public schools, supermarkets, correctional facilities, hospitals, and nursing homes. This tool has proven effective to link the mutual interests of the farmer and food waste sources.

Plant Residues on the Farm

Most farm-based anaerobic digesters in Europe use co-digestion of animal manures with organic wastes, such as crop residues or energy crops. In Germany, for example, a high percentage (over 90%) of agro-biogas plants use co-digestion. Organic materials of over 30 byproducts and wastes are used, but energy crops and crop residues are favored relative to off-farm wastes to avoid the cost of transportation to the farm. Most crops can be co-digested with manure, with maize and grasses as most important examples. The advantages of maize and

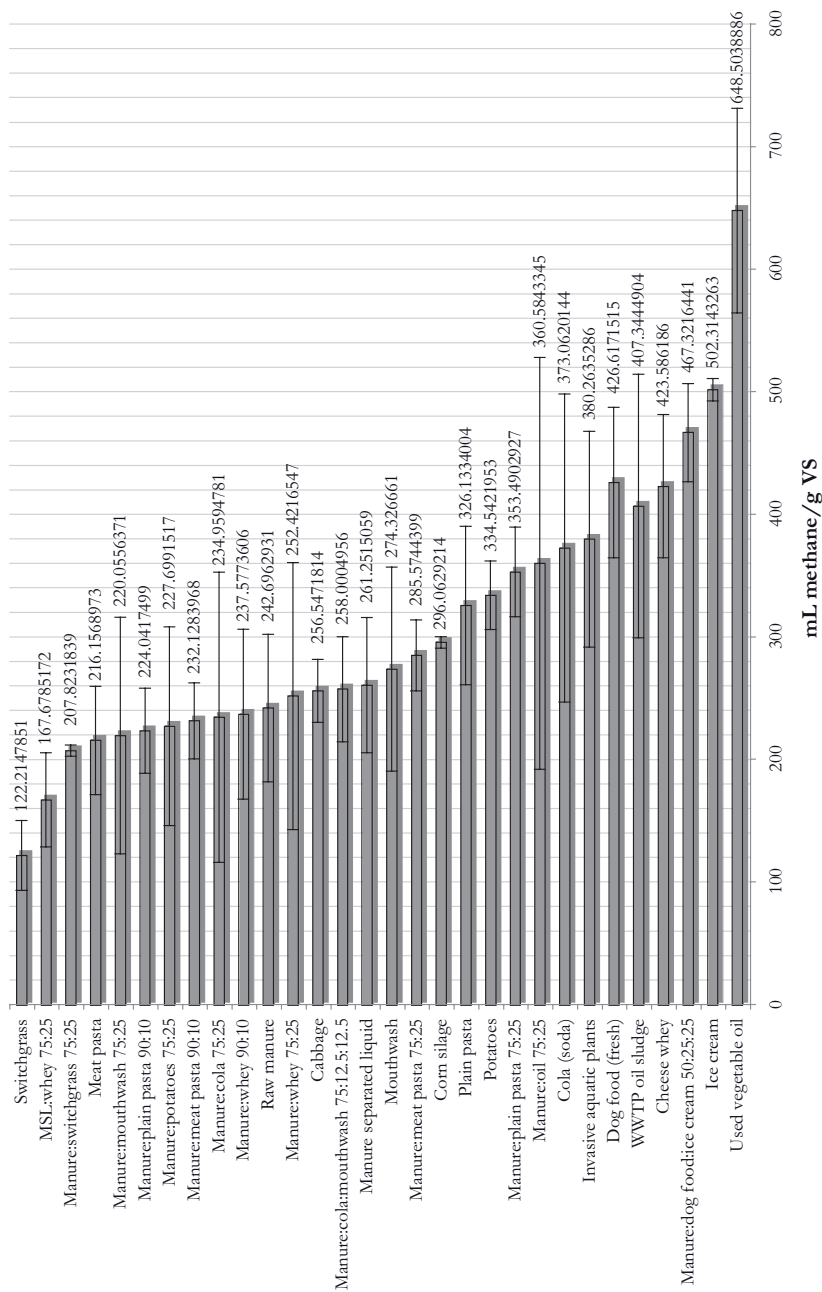


Figure 4.1. Summary of the normalized methane yields (values next to the bars) for some 30 individual substrates and substrate mixtures analyzed using the biochemical methane potential (BMP) assay. Error bars represent the standard deviation of the normalized methane yield for each substrate (adapted from Labatut and Scott [2008]).

grasses are the relatively high biogas yield per hectare for maize and the low energy input required for grasses. Both maize and grasses can be readily stored to provide a year-long input for digesters (Chynoweth et al. 1993; Gunaseelan 1997; Lewandowski et al. 2003; Lehtomaki et al. 2008). The disadvantage may be the very long digestion residence times required. Ensilage of whole crop maize, for example, is one potentially important process to conserve organic material and provide for pretreatment of feedstock (Driehuis et al. 1999).

The results of some of these studies show that up to 3000–5000 m³ of methane can be generated from a hectare of cultivated energy crops (Lehtomaki et al. 2008). Most crops have methane potentials in the range of 0.25 to 0.4 L CH₄/g VS_{added}. In general, the methane potential increased with crop maturity. Anaerobic digestion of dairy manure with crop materials (sugar beet tops, grass silage, and oat straw) in completely stirred tank reactors (CSTRs) was feasible with up to 40% VS for the feedstock (60% for manure; Chynoweth et al. 1993; Lewandowski et al. 2003; Bouallagur et al. 2005). This is in agreement with Lehtomaki et al. (2008), who obtained specific methane yields between 0.21 and 0.27 L CH₄/g VS_{added} for straw, sugar beet tops, and grass as feedstocks with cow manure (30% and 70% based on VS, respectively) in a co-digestion approach.

Practical Studies to Optimize Methane Generation from Agricultural Wastes

Low-Rate versus High-Rate Treatment of Animal Waste

Low-Rate Animal Waste Digestion

Many full-scale animal waste digester systems are low-rate anaerobic digesters, such as covered anaerobic lagoons, plug-flow digesters, and CSTRs. A typical VS loading rate (VSLR) for an unheated covered anaerobic lagoon is 0.24 g/L/day with a hydraulic retention time (HRT) of 65 days (Cheng et al. 1999). By feeding dairy waste at a VS concentration of 5 g/L in a 4.5-L low-rate bioreactor, we achieved a maximum VSLR in a heated (34°C) and completely stirred anaerobic digester (semi-batch fed system) of 3.5 g/L/day and a minimum HRT and sludge retention time of 15 days (Hoffmann et al. 2008). A typical VSLR and HRT for a heated (35°C) CSTR-fed swine waste at a concentration of 20 gVS/L are 1.4 g/L/day and 15 days, respectively. Design parameters for a completely stirred system are dependent on the VS concentration in the influent, the operating temperatures, and composition of the biomass in the waste. Cow and swine wastes have a considerably different composition and require different operating conditions to achieve a stable and sufficient treatment performance. In addition, each farm is different, for example, in how the choice of bedding material for dairy cows will affect the required operating conditions (NRCS 1996).

High-Rate Animal Waste Digestion

High-rate anaerobic treatment systems rely on biomass settling or membrane technology to achieve longer solids retention times compared with HRTs for satisfying performance under high volumetric loading rates. This results in smaller reactor volumes, and thus lower construction costs than low-rate systems, because the volume of anaerobic digesters is sized based on the HRT, while the performance of all digesters is dependent on the sludge retention time. Full-scale high-rate anaerobic digesters have shown excellent and stable performance for over 30 years, but have been used mainly for low-solids industrial and domestic

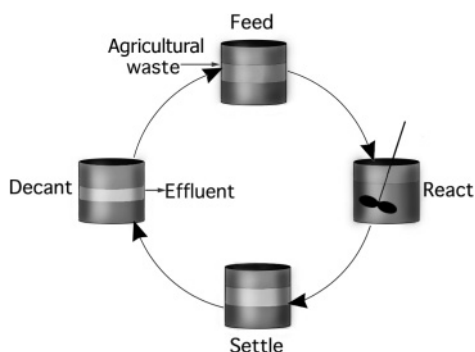


Figure 4.2. Anaerobic sequencing batch reactor (ASBR) cycle. One reactor vessel sequences through time by four steps: (1) feed step during which waste is introduced; (2) react step during which intermittent mixing provides contact with substrate and biomass; (3) settle step during which biomass is concentrated at the bottom of the reactor by switching off mixing; and (4) decant step during which the treated waste is removed. Then, the cycle is repeated.

wastewater treatment (Lettinga 1995; Hulshoff Pol et al. 1997; Seghezzo et al. 1998). For high-solids wastewater or slurries, such as diluted swine waste, existing technologies (e.g., up-flow anaerobic sludge blanket and its derivatives) showed problems with solids separation and solids accumulation in the reactor (Zeeman et al. 1997; Elmitwalli et al. 1999; Kalogo and Verstraete 1999). The anaerobic sequencing batch reactor (ASBR) was developed to treat a high-solid influent with a high-rate technology (Figure 4.2; Dague et al. 1970; Dague and Pidaparti 1992). Both the ASBR and an alternative technology—the plug-flow anaerobic baffled reactor—have made it possible to treat high-solids waste (e.g., animal waste) by high-rate systems (Zhang et al. 1997; Boopathy 1998).

ASBRs

Our lab has experience with treatment of swine waste with ASBR technology, and therefore we will use this technology as an example of a high-rate anaerobic digester system on the farm (Garcia and Angenent 2009). The ASBR is a single-vessel system that requires no feed-distribution and gas-solids-separation systems, which simplify its design (Figure 4.2). However, intermittent mixing is needed to provide sufficient contact between substrate and biomass, distribute heat, and prevent scaling. A settling step before decanting effluent is instrumental to facilitate high biomass levels and long sludge retention times (internal settling; Sung and Dague 1995). Developing a well-settling biomass, while not disturbing bioflocculation, was accomplished by mixing ASBRs intermittently (e.g., 2 minutes of mixing every hour for a laboratory-scale bioreactor; Sung and Dague 1995; Zhang et al. 1997). Development of a well-settling biomass from poorly settling inocula, however, requires long start-up periods. Several laboratory-scale studies and one full-scale study have been performed to investigate swine waste treatment with ASBRs (Dague and Pidaparti 1992; Zhang et al. 1997; Angenent et al. 2002b, 2008). Indeed, for efficient swine waste treatment at the design volumetric loading rates, the start-up period was longer than 6 months.

The choice of inoculum influences the start-up period of ASBRs. This became apparent in a study with two 5-L ASBRs that treated diluted swine waste with a VS level of 20 g VS/L (Angenent et al. 2002a). When swine lagoon sludge with a very well-settling ability (sludge volume index [SVI] < 10 mL/g total solids [TS]) was used as an inoculum, the period to

achieve a design VSLR of 4 g/L/day was 250 days due to the fact that biomass levels in the bioreactor remained around 20 gVS/L during the initial 50 days of the operating period (Figure 4.3a,b). The operating period to achieve this design loading rate was 100 days longer when poorly settling anaerobic digester sludge from a CSTR treating waste activated sludge was used as an inoculum (SVI was ~56 mL/gTS). Biomass washout in the ASBR with this sludge decreased the biomass levels in the reactor from 20 to 10 gVS/L during the first 50 days of the operating period (Figure 4.3b). At the end of the study after a 1-year operating period, the settleability of the biomass in both reactors was identical (17 mL/gTS), showing that ASBRs select for a well-settling biomass (Angenent et al. 2002a).

Besides developing a well-settling biomass, the concentration of methanogens in the reactor biomass may also need to be increased to obtain high volumetric loading rates. Timur and Öztürk (1999) reported that an ASBR showed favorable performance during the treatment of landfill leachate. In addition, these researchers reported that the volumetric methane production rate (VMPR) increased linearly with the volumetric loading rate to achieve a constant methane yield over the operating period during which a stable performance was reported (Timur and Öztürk 1999), which we also observed for animal wastes (Angenent et al. 2002b; Angenent et al. 2008; Hoffmann et al. 2008; Garcia and Angenent 2009). Timur and Öztürk (1999) observed an enrichment of methanogens over the operating period, with an increasing specific methane production rate (in L of methane per g volatile suspended solids [VSS] per day) to keep up with the increase in the food to microorganism (F/M) ratio. This showed that the biomass in the ASBR became more active in terms of methane production per amount of biomass present over the operating time and that a considerable start-up period was required before the ASBR could manage volumetric loading rates of up to 10 gCOD/L/day (Timur and Öztürk 1997). We have shown higher concentrations of methanogenic small-subunit ribosomal RNA (rRNA) in ASBRs treating swine waste at the higher VMPR under stable reactor performance compared with the lower methane production rate (Figure 4.4). Therefore, anaerobic digesters select for a biomass that is more active in methane production to sustain higher loading rates (Angenent et al. 2002a).

In summary, both the development of a well-settling biomass and an increase in the concentration of methanogens necessitates a long start-up period for the ASBR. In our work with ASBR treatment of diluted swine waste at 25–35°C, the investment of a relatively long start-up period, however, ensured an operation at a much shorter HRT of 5 days compared with a 15-day HRT for low-rate anaerobic digesters (Angenent et al. 2008; Garcia and Angenent 2009). This shorter HRT directly results in a 66% reduction in reactor volume. At the relatively low HRT of 5 days for a 5-L ASBR treating swine waste at a concentration of 2 gVS/L and 25°C, the methane yield was 0.47 L methane per g VS fed with an ~52% VS removal efficiency at a VSLR of 4 g/L/day (Angenent et al. 2008). This yield was close to the ultimate methane yield (i.e., methane yield at an infinite HRT), which was estimated to be between 0.44 and 0.52 L CH₄/g VS fed for waste from swine fed a corn-based diet (Chen 1983), and shows that the ASBR performance is suitable even under relatively low HRTs.

In a subsequent study, we operated the same ASBRs under similar environmental conditions, but with swine waste from a different farm. We achieved a lower methane yield of 0.31 L methane/g VS fed (Garcia and Angenent 2009), and this was not caused by an inferior ASBR performance compared with the study described earlier (a 51% and 52% VS removal efficiency at a VSLR of 4 g/L/day for the Angenent et al. [2008] and Garcia and Angenent [2009] studies, respectively). Instead, the large difference in methane yields for both studies was probably due to variations in the composition of swine waste. This shows that it is very difficult to predict the methane yields due to the large variations in conditions at the

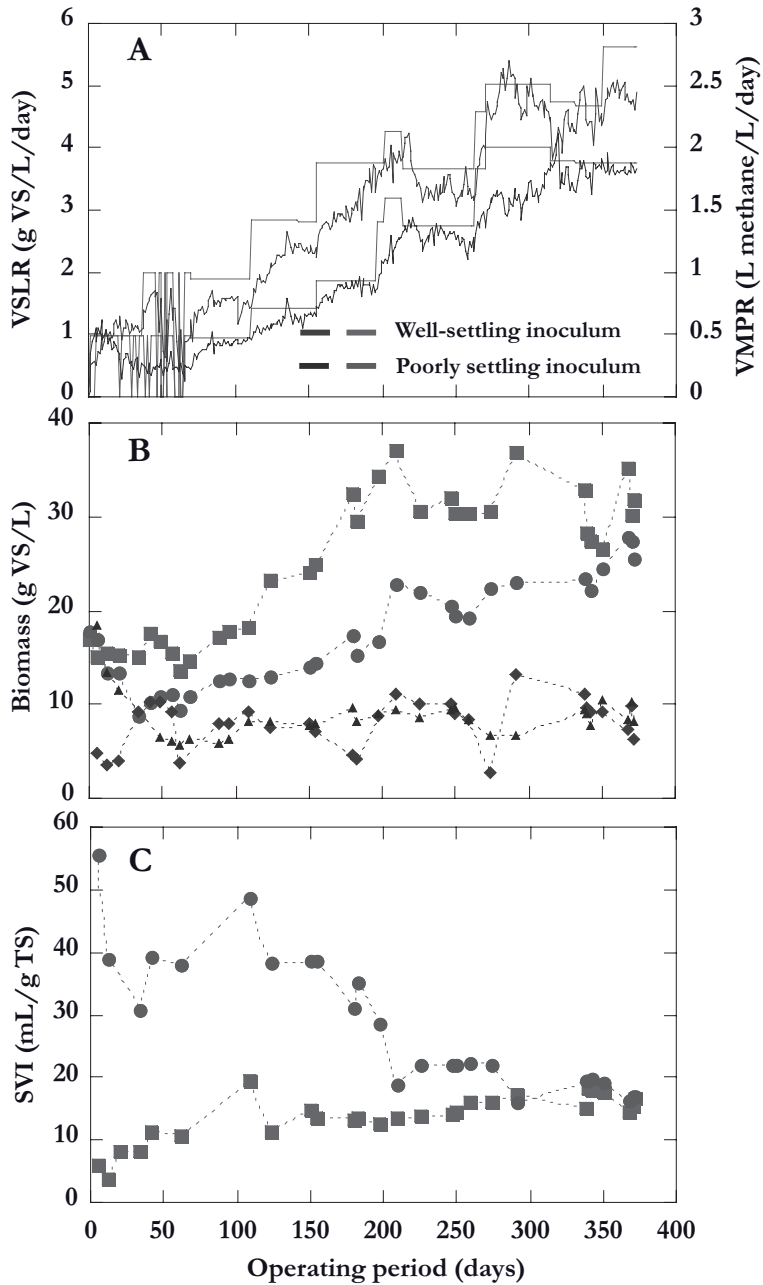


Figure 4.3. Experimental data from two side-by-side ASBRs with different inocula over an operating period of ~1 year. A. Volumetric volatile solids loading rate (straight lines) and volumetric methane production rate (zigzag lines) for well-settling (top) and poorly settling (bottom) inoculum; B. biomass concentration in reactors (■, well-settling; ●, poorly settling inoculum) and effluent (◆, well-settling; ▲, poorly settling inoculum); and C. sludge volume index (■, well-settling; ●, poorly settling inoculum) (adapted from Angenent et al. [2002a]).

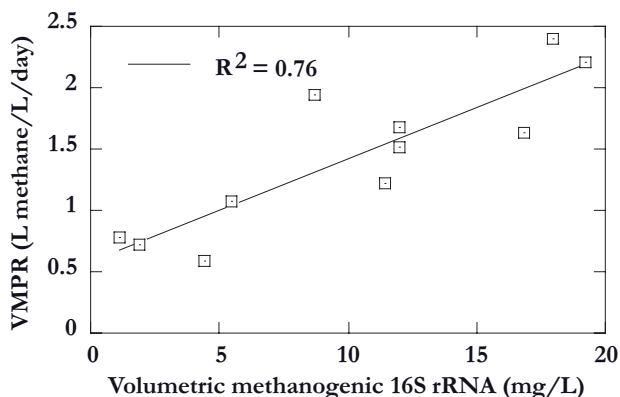


Figure 4.4. Correlation between the volumetric methane production rate and the amount of total methanogenic 16S rRNA present per reactor volume in the ASBR that was inoculated with poorly settling biomass. This result indicates that methanogens must be enriched in the biomass over long operating periods to achieve preferred high volumetric methane production rates (adapted from Angenent et al. [2002a]).

farm, including farm practices, such as animal feed composition, antibiotic use, manure collection, manure storage period before transfer into the digester, water consumption, and cleaning habits.

Mixing in Digesters

Conflicting Reports on Mixing in Digesters

The literature contains conflicting information regarding the mixing intensity and duration that is necessary in digesters to treat a waste with a high concentration of VS. In general, workers agree that mixing is necessary to enhance substrate contact with the microbial community, improve pH and temperature uniformity, prevent stratification and scum accumulation, facilitate the removal of biogas, and aid in particle size reduction (Stafford 1981). However, mixing can be performed intermittently or continuously at different intensities (Hansen et al. 1999; Karim et al. 2005). On the one hand, Dague et al. (1970) showed that intermittent mixing resulted in an increased biogas production and increased COD and VS reduction efficiencies due to enhanced bioflocculation (settling) compared with continuous mixing. In addition, Stroot et al. (2001) and McMahon et al. (2001) reported that minimally mixed digesters demonstrated a much more stable operation than digesters that were continuously and vigorously mixed. They postulated that vigorous, continuous mixing inhibited relationships between syntrophic bacteria and their methanogenic partners, possibly by disrupting the spatial juxtaposition between these organisms. On the other hand, Lanting (2003) and Muller et al. (2007) showed that high mixing intensities results in particle size reduction and diffusion limitation reduction, which increased processing capacity for a digester treating waste-activated sludge. The choice in mixing intensity and duration is important, because continuous and vigorous mixing may significantly increase the necessary energy (i.e., electricity) input for the digestion system, and thus reduce the net energy balance ratio. To shed light on the advantages and disadvantages of mixing intensity and duration for animal waste digestion, we conducted two different studies: one with swine waste in high-rate ASBRs,

which is described in the Mixing in a High-Rate System Treating Swine Waste section (Angenent et al. 2001), and the other with dairy waste in the low-rate completely stirred anaerobic digester, which is described in the Mixing in a Low-Rate System Treating Dairy Waste section (Hoffmann et al. 2008). We found that low-rate and high-rate reactors are fundamentally different in regard to mixing requirements, and that the use of different reactor types and configurations may explain the discrepancies in the digester literature in regard to mixing characteristics (Hoffmann et al. 2008).

Mixing in a High-Rate System Treating Swine Waste

We operated two lab-scale ASBR systems in parallel and the mixing duration and intensity were changed in one bioreactor from gentle, intermittent mixing to gentle, continuous mixing to vigorous, continuous mixing, while the other bioreactor (control) was operated throughout the experiment with gentle, intermittent mixing (Angenent et al. 2001). The substrate for this study was diluted swine waste (20 gVS/L) at a volumetric loading rate of ~4 gVS/L/day. The inoculum of the ASBRs was from previously operated ASBRs that contained well-acclimated biomass with excellent settling characteristics (Angenent et al. 2002a), and therefore the start-up period was less than 10 days (Figure 4.5A).

Mixing Duration

When on day 23 of the operating period the mixing duration was changed from intermittent to continuous mixing, continuous mixing resulted in a higher biomass level in the effluent (12 gVS/L) compared with an effluent biomass level of 8 gVS/L for the control reactor (Figure 4.5B) due to an increase in the SVI of the washed out biomass from 27 to 37 mL/gTS (the SVI of the biomass in the reactor increased from 15 to 19 mL/gTS; Angenent et al. 2001). Even though some biomass loss became apparent, the biomass concentration in the reactor stabilized to a level of 22 gVS/L compared with 29 gVS/L (Figure 4.5B) and stable reactor conditions prevailed with similar soluble COD and total volatile fatty acid (VFA) concentrations compared with the control reactor. After a steady-state biomass concentration was achieved in the bioreactors, the VMPR was slightly lower for the continuously mixed bioreactor compared with the intermittently mixed control (1.7 vs. 1.9 L/L/day; Figure 4.5A) due to a slightly lower VS removal efficiency in the continuously mixed bioreactor (related to a higher VS level in the effluent). In addition, the relative 16S rRNA levels for the predominant acetoclastic methanogens *Methanosaeta concilii* and the predominant hydrogenotrophic methanogens from the order Methanomicrobiales had only decreased somewhat in the continuously mixed digester (but had stabilized) compared with the control reactor (Figure 4.5C,D). Thus, the change in mixing duration for the ASBR deteriorated the performance slightly with lower VS removal efficiencies, but did not jeopardize the methanogenic community structure or the stability. This result shows that intermittent gentle mixing is advantageous, and indicates that continuous mixing is not required for efficient conversion in high-rate anaerobic digesters, and, in fact, reduced the methane yield. Noteworthy is that during the gentle mixing period the concentrations of the methanogenic populations were much lower in the effluent biomass compared with the reactor biomass (Figure 4.5C,D). This is another advantage of high-rate anaerobic digester systems.

During the 24-hour feeding cycle (ASBRs were fed instantly at $t = 0$), the shape of the cumulative biogas production curves were similar between the intermittently and continuously mixed bioreactors at gentle mixing conditions with a lower slope at the end of the cycle (Figure 4.6A). This confirms the previous conclusion that reactor stability was not

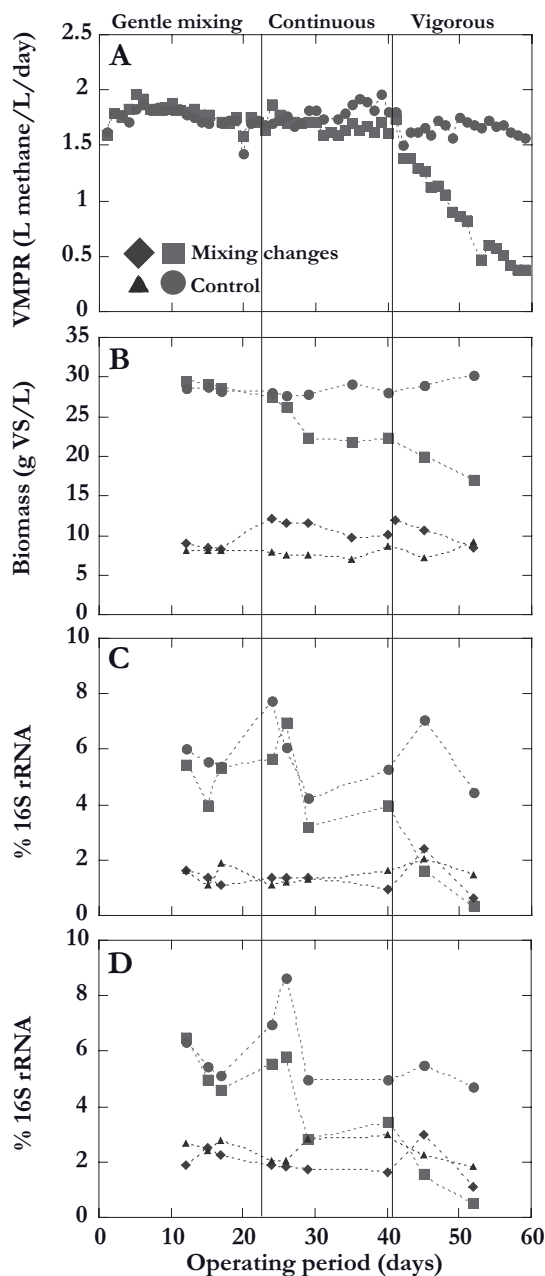


Figure 4.5. Experimental data from two side-by-side ASBRs operated under different mixing conditions. The biomass had been acclimated during the 1-year inoculum study (Angenent et al. 2002a). A. Volumetric methane production rate; B. Biomass concentration in reactors (■, ●) and effluent (◆, ▲); C. Relative 16S rRNA levels for the methanogen *Methanosaeta concilii*; and D. Relative 16S rRNA levels for the methanogens in the order Methanomicrobiales (adapted from Angenent et al. [2001]).

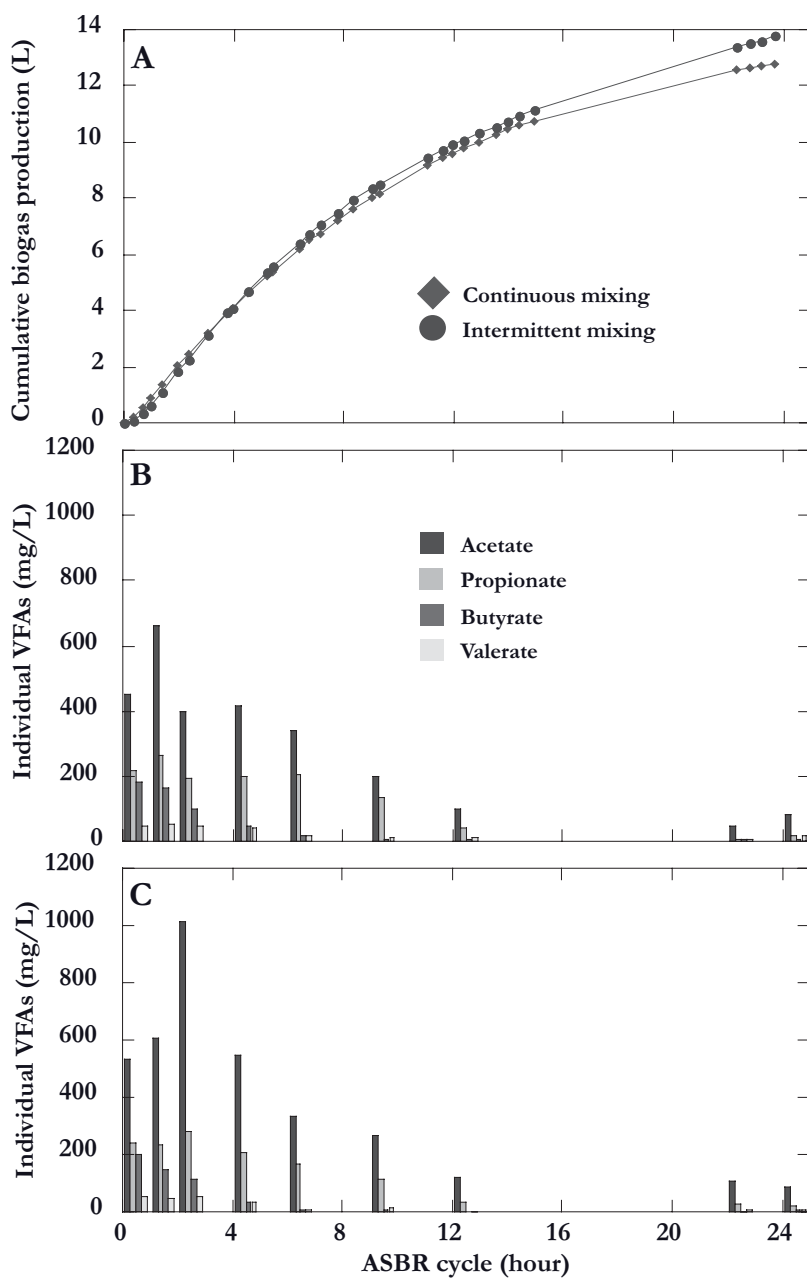


Figure 4.6. Experimental data obtained during a 24-hour ASBR cycle from two side-by-side reactors with the only change being mixing duration (Angenent et al. 2001). A. Cumulative biogas production during one ASBR cycle for the continuously mixed ASBR and the intermittently mixed ASBR (control); B. Individual volatile fatty acids concentration during one cycle for the continuously mixed ASBR; and C. Individual volatile fatty acids concentration during one cycle for the intermittently mixed ASBR (control).

jeopardized during continuous/gentle mixing. The shapes of the cumulative production in both bioreactors show a lag period with a relatively low biogas production rate just after feeding and then a maximum rate (i.e., slope) between 1 and 3 hours after feeding, corresponding to a higher acetate concentration in the mixed liquor between 600 and 1000 mg/L (Figure 4.6B,C). The lowest biogas production rates were observed at the end of the 24-hour cycle when the acetate concentration was ~100 mg/L in both systems (Figure 4.6B,C). Some differences were noticed between the cumulative curves of the two bioreactors (no difference was observed when both reactors were intermittently and gently mixed), because the rate of biogas production was faster ~1 hour after feeding for the continuously mixed bioreactor versus the control bioreactor (Figure 4.6A), indicating minor diffusion limitations in the intermittently mixed reactor. After 4 hours in the cycle, however, the cumulative biogas production in the intermittently mixed bioreactor had caught up with the continuously mixed bioreactor due to a higher maximum rate between 1 and 3 hours after feeding swine waste (Figure 4.6A). The maximum biogas production rates (slope) matched the location of the maximum acetate concentration, which occurred for the continuously mixed reactor after ~1 hour (660 mg/L) and for the intermittently mixed control reactor after ~2 hours (1015 mg/L; Figure 4.6B,C). This result shows that mixing duration affects the function of the anaerobic food web for high-rate anaerobic digestion.

Mixing Intensity

The change (on day 40 of the operating period) from gentle continuous mixing by biogas recirculation to vigorous continuous mixing by liquid recirculation with a peristaltic pump showed a very different outcome. The vigorously mixed bioreactor showed an immediate and steady decline in the VMPRs (Figure 4.5A). Feeding had to be terminated on day 53 to prevent complete digester failure because the VFA levels in the effluent had increased from an average of 256.3 mg acetate/L on day 40 to 2846 mg acetate/L on day 52. Biomass washout was partly to blame, because biomass levels in the effluent had increased from 10 g VS/L on day 40 to 12 g VS/L on day 41, resulting in a steady loss in VS biomass in the vigorously mixed bioreactor (the data for the control bioreactor remained constant; Figure 4.5B). In addition, slot-blot hybridization techniques showed that a severe loss in the concentration of the predominant methanogens was the result of the change in mixing intensity. That is, the relative 16S rRNA levels for *M. concilii* and for the order Methanomicrobiales decreased to levels lower than 1% on day 52 of the operating period (Figure 4.5C,D). This result supports the hypothesis postulated by Stroot et al. (2001) and McMahon et al. (2001) that vigorous, continuous mixing inhibited relationships between syntrophs and their methanogenic partners by destroying their juxtaposed position. In addition, it is also in agreement with Hoffmann et al. (2008), who found that the filamentous concentrations of *M. concilii* was negatively affected by increased mixing intensities. Thus, vigorous mixing decreased the concentration of methanogens, which had been slowly built up during the long start-up periods (described in the ASBRs section). With the loss of methanogenic activity, the high-rate system became severely overloaded, and combined with a declining biomass concentration, unstable conditions emerged within weeks.

Mixing in a Low-Rate System Treating Dairy Waste

An opposite result for different mixing intensities emerged for low-rate, completely stirred anaerobic digesters that do not rely on biomass settling for advanced VSLR—mixing intensity

did not affect long-term reactor performance. For this study with low-rate systems, microbial flocs had not developed in any of the four lab-scale bioreactors, which were continuously mixed with an impeller at different rotational speeds of 50, 250, 500, and 1500 revolutions per minute (RPM; Hoffmann et al. 2008). In the system with continuous mixing, diffusion limitations for hydrogen and acetate were overcome and this circumvented the requirement for a juxtaposed position of bacteria and their syntrophic methanogenic partner in flocs. Thus, a higher methanogenic activity of biomass was not dependent on floc formation and a juxtaposed position could, therefore, not be destroyed by a more intense mixing scheme. During the initial start-up period, however, the performance of the most intensely mixed digester was hampered. Based on this result, we concluded that mixing intensity should be low during initial start-ups. In addition, we found that the acetoclastic methanogenic community structure was affected by the mixing intensities applied to the bioreactors. An inverse correlation between mixing intensity and relative 16S rRNA levels for *M. concilii* was found. At the higher mixing intensities, acetate was converted to methane by *Methanosarcina* spp., while this was converted by *M. concilii* at the lower mixing intensities (Hoffmann et al. 2008). On one hand, this may be important in regard to long-term stability because *Methanosarcina* spp. (with a higher substrate utilization rate at elevated acetate concentrations) can handle shock loads better than *Methanosaeta* spp. On the other hand, the affinity for acetate is higher for *Methanosaeta* spp. than *Methanosarcina* spp., resulting in lower effluent acetate concentration, and therefore higher methane yields.

Ammonia Toxicity

For the agricultural wastes that are characterized with high protein and/or urea levels, ammonia toxicity to the bacterial and methanogenic populations in the undefined mixed cultures in anaerobic digestions can hamper reactor performance. (Ammonium is the final digestion product for nitrogen-containing polymers, such as proteins.) Ammonia toxicity in anaerobic digestion, therefore, has been studied in depth over the last 30 years (Velsen 1979; Braun et al. 1981; De Baere et al. 1984; Hashimoto 1986; Koster and Koomen 1988; Koster and Lettinga 1988; Bhattacharya and Parkin 1989; Robbins et al. 1989; Poggi-Varaldo et al. 1991; Troyer 1997; Lay et al. 1998; Lu et al. 2008). Some of these papers documented maximum concentrations of total ammonium-N (NH_3 and NH_4^+) and free ammonia (NH_3), which is the inhibiting species, for pure cultures of methanogenic populations (Sprott and Patel 1986; Koster and Koomen 1988; Steinhaus et al. 2007). The general consensus in the literature seems to indicate that hydrogenotrophic methanogens are less sensitive to high free ammonia concentrations compared with acetoclastic methanogens (Sprott and Patel 1986; Angelidaki and Ahring 1993). It is, therefore, not surprising that under high ammonia concentration an alternative pathway that includes acetate oxidation by bacteria and subsequent hydrogen utilization by methanogens emerges in farm-based digesters without the need for acetoclastic methanogens (Schnürer et al. 1994, 1996; Angenent et al. 2002b). Angenent et al. (2002b) showed that the relative 16S rRNA levels for hydrogenotrophic methanogens of the order *Methanomicrobiales* increased from 2.3% to 7% with increasing total ammonia-N concentrations of 2000 to 3600 mg/L, whereas the relative 16S rRNA levels for acetoclastic methanogens in the genus *Methanosarcina* decreased from 3.8% to 1.2%. Others have observed that acetoclastic methanogens from the family *Methanosarcinaceae* tend to group themselves in a cluster shape when a relatively high total ammonium-N concentration is present to protect themselves from high free ammonia concentrations (Calli et al. 2005a,b). Thus, several mechanisms to sustain an efficient microbial community function under high

free ammonia concentrations may be present, resulting in a diverse methanogenic population even when the total ammonium or free ammonia concentration in the farm-based digester has exceeded the maximum levels reported in the literature.

In a recent study, we have operated four identical ASBR systems-fed swine waste at a VS concentration of 20 g/L for close to a 1000-day operating period (Garcia and Angenent 2009). Several operating changes, including ammonia concentration and temperature increases, were made to study the effect on the interactions between temperature and ammonia inhibition on reactor performance. During period 1 (day 0–378), the methane yield was 0.31 L CH₄/g VS for all digesters (with no statistical differences among them) at a temperature and total ammonium-N levels of 25°C and ~1200 mg ammonium-N/L, respectively. During period 2 (day 379–745), the methane yield at 25°C decreased by 45% when total ammonium-N and free ammonia-N were increased in two of the four ASBRs to levels >4000 mg/L and >80 mg/L, respectively. During period 3 (day 746–988), this relative inhibition was reduced from 45% to 13% compared with the low-ammonia control reactors when the operating temperature was increased from 25°C to 35°C (while the free ammonia levels increased from ~100 to ~250 mg ammonia-N/L because of the temperature increase—chemical equilibrium change). The 10°C increase in temperature doubled the kinetic constant for methanogenesis, which overwhelmed the elevated toxicity effects caused by the increasing concentration of free ammonia, resulting in better reactor performances. This was an unexpected result (for the authors) because the literature predicts that methanogenesis would become severely inhibited with free ammonia concentrations of ~250 mg-N/L at a higher (35°C) operating temperature (Koster and Lettinga 1984; Sprott and Patel 1986; Koster and Koomen 1988). Based on the chemical equilibrium change, we had predicted pronounced methanogenic inhibition at temperatures higher than 25°C. However, based on a long-term study with methanogenic food webs, it is clear that the farmer/operator may alleviate ammonia toxicity by increasing the operating temperature within the mesophilic range. Even a temperature increase from 30–35°C to 38–39°C may increase digester performance at elevated ammonia-N concentrations (Garcia and Angenent 2009).

Thermophilic Digestion

Even though on many farms increasing the temperature of vast quantities of cold influent streams to thermophilic (55°C) levels may not be feasible, thermophilic anaerobic digestion has been important for energy recovery from Danish agricultural wastes (Ahring et al. 1992; Angelidaki and Ahring 1992, 1993, 1994; Hansen et al. 1999; Ahring et al. 2001). The advantage of thermophilic anaerobic digestion lies in the superior kinetic rates at higher temperatures because of improved hydrolysis rates and methane yields (Vandevoorde and Verstraete 1987; Mackie and Bryant 1995; Sung and Santha 2003). Therefore, thermophilic digesters have been found to improve solids destruction over mesophilic digesters (35–37°C; Angelidaki and Ahring 1994; Sung and Santha 2003). Thermophilic digestion of animal wastes would also allow for sufficient pathogen destruction and the generation of biosolids that can easily be dispersed into the environment (Han and Dague 1997; Welper et al. 1997). However, protein-rich wastewater treatment at thermophilic conditions has shown to be problematic. Because of digestion of proteins into the end-product ammonia, higher levels of free ammonia at thermophilic compared with mesophilic temperatures (with similar total ammonia concentrations) have inhibited methanogenesis and caused unstable performances (Zeeman et al. 1985; Angelidaki and Ahring 1993; Lettinga 1995; Zitomer et al. 2005; Bocher et al. 2008). Acetate oxidation at thermophilic conditions may alleviate these unstable

conditions somewhat because thermophilic hydrogenotrophic methanogens can tolerate higher levels of ammonia than mesophilic hydrogenotrophic methanogens (Hendriksen and Ahring 1991) and we already know that in general the hydrogenotrophic methanogens can tolerate ammonia better than acetoclastic methanogens (Sprott and Patel 1986). Despite possible acetate oxidation, ammonia inhibition at thermophilic conditions remains a real problem. In Denmark, mixing carbon-rich waste streams with protein-rich animal wastes to “dilute” the concentrations of the fermentation end-product ammonia has solved this problem. A report by Lindorfer et al. (2008) investigated the increase in operating temperature beyond mesophilic temperatures by self-heating anaerobic digesters fed with energy crops. They found that short temperature pulses and addition of acclimated biomass can circumvent negative effects of high free ammonia concentrations to digester performance during periods of increasing temperatures.

High-Solids Digestion

Thus far, we have mostly discussed anaerobic digestion of diluted farm-based wastes (~3%–8% TS in the reactor), such as slurries of swine waste and dairy manure. However, “dry fermentation” has found a niche in the bioenergy industry as well (~18%–35% TS in the reactor), especially when energy crops are used as feedstock. These systems are most often operated at thermophilic conditions to take advantage of the superior hydrolysis rates (Richards et al. 1991; De Baere 2000). The advantage of high-solids digestion compared with low-solids digestion is that high-solids digestion requires smaller reactor volumes than low-solids digestion due to high VMPRs. However, possible toxicity of metals and ammonia must be taken into consideration (Jewell et al. 1993). Dry fermentation has also been referred to as anaerobic composting, dry digestion, or high-solids digestion (Jewell et al. 1993; Chyi and Dague 1994; De Baere 2000). Systems that are operated with a TS content between ~10% and 18% have been referred to as semidry digestion (Mata-Alvarez et al. 1993). Numerous pilot-scale and full-scale plants with different designs have been built and operated, and excellent performances have been reported. For example, a volumetric biogas production rate of 9.2 L/L/day (this means that 10 times as much biogas is produced than the volume of the digester itself) was reported for a full-scale dry anaerobic composting (DRANCO) process for which the TS in the system averaged 31.3% (De Baere 2000).

Full-Scale Experiences

Anaerobic Digestion in China

Anaerobic digestion is a scalable process, which is often overlooked in the United States and is frequently stated to be economically viable only for large farms. Dairy farms, for instance, are thought to be economical for ~500 cows or more. The scalability feature is particularly evident in China, where by 2005 more than 18 million household digesters have been installed providing 7 billion m³ of biogas per year (van Nes 2006). A common operating system is a small inground digester fed with swine waste from a few pigs and human waste. The generated biogas is used in the household for cooking and lighting. The Ministry of Agriculture (MOA) aims to increase the number to 27 million household digesters by 2010. In addition to the household digesters, in 2005 there were almost 3500 medium- and large-scale biogas digesters at livestock and poultry farms (van Nes 2006; Figure 4.7).



Figure 4.7. Chinese mid-sized anaerobic digester system near Shanghai.

Anaerobic Digestion in Germany

In Europe, the greatest adoption of anaerobic digestion has taken place in Germany. The Committee on Agriculture and Rural Development of the European Parliament (2004–2009) developed a report on Sustainable Agriculture and Biogas, which stated that “A Need for Review of EU-Legislation acknowledges biogas as a vital energy resource to contribute to sustainable economic, agricultural and rural development and environmental protection with the strong recommendation that the EU exploit the very large potential for biogas” (EU 2008). A recent German report claimed that Germany could produce more biogas by 2020 than all of EU’s current natural gas imports from Russia (Burgermeister 2008).

At the end of 2007, Germany had ~3700 biogas plants with a total electric capacity of 1.25 GW in operation. Most of the new biogas plants have an electrical capacity between 400–800 kW, which was estimated by the German Energy Agency (DENA 2008). Energy crops make up a substantial portion of the substrate mixture with manure substrate at 50% or less. Germany is growing energy crops on ~1.3 million ha (~11.4 % of its arable land). Although there are a number of large biogas digesters at wastewater treatment plants, landfill installations, and industrial bio-waste processing plants, the greatest volume of biogas is produced on farms and large co-digestion biogas plants. Noteworthy is that in Germany (and also in Austria where a similar biogas revolution has taken place) some automobiles run on biogas.

In Germany almost all agro-biogas plants use co-digestion with over 30 different organic byproducts and wastes. The drivers for the extensive biogas plants and electrical generating capacity are the implementation of: (1) the Renewable Energy Resources Act; (2) guaranteed purchase of electricity produced from biogas at preferential rates for 20 years; (3) bonuses for electricity produced from renewable resources; (4) CHP systems; and (5) new technologies.

The confluence of renewable energy and economic and rural development has led to biogas-driven energy for sustainable rural communities. A good example is the village of Jühnde, where a biogas plant of 700 kW provides all of the required electricity and most of

the thermal energy for ~750 residents. Home heating is supplied via a district hot water grid of ~3.3 miles (Fangmeier 2008). The feedstock for the digester is derived from manure of nine farms and from energy crops (i.e., maize, grasses, and wheat).

Anaerobic Digestion in Denmark

Biogas generation from agricultural wastes was first introduced in Denmark in the late 1970s during the first energy crisis. Although not as widespread as on farms in Germany, where many small farms have digesters, Denmark has three categories of digester: (1) 20 community (centralized) biogas plants ranging from 540 to 7500 m³ in volume and fed with animal and industrial wastes, which provide electricity to the grid and heat for houses (Figure 4.8); (2) eight large farm biogas plants with CHP, but without co-digestion; and (3) 18 farm biogas digesters operated at thermophilic conditions in the capacity range of 150–800 m³ with co-digestion and CHP (Al Seadi and Holm-Nielsen 2000; Sannaa 2004).

Nearly all community biogas plants are performing co-digestion, partly for increased biogas production, but also for “tipping fees” from suppliers of organic wastes. The average community digester obtains its income from three sources: electricity, heat, and tipping fees from industries. In general, such plants get ~20% to over 50% of their wastes from nonagricultural suppliers (e.g., slaughter houses, fish processing, pharmaceutical industry, hospital kitchens, hotels). Drivers for anaerobic digesters in Denmark have been investment grants to help capital costs (~20%), long-term loans at low interest rates, a requirement for a 9-month storage for untreated manure slurry, favorable prices for biogas-produced electricity, opportunities for district heating, and demonstration and research programs.

Anaerobic Digestion in the United States

Reduction of odors and water pollution as well as potential energy generation (CHP) on large livestock operations have stimulated increased interest in anaerobic digestion systems. Since the mid-1970s, interest in methane generation technology in the United States has varied. Early anaerobic digestion in the United States had been farm-based with primary efforts to develop appropriate technology, which would require low initial capital cost, low operating



Figure 4.8. Ribe Biogas Ltd. centralized biogas plant in Ribe, Denmark.

Table 4.2. Estimation of digesters on U.S. life-stock farms in December 2008 (adapted from the U.S. EPA-AgSTAR [2008]).

Farm Type	Number of Digester Projects
Dairy	93
Swine	20
Caged layer	3
Duck	2
Broiler	1
Beef	1
Mixed	1

costs, low maintenance, little operator time, and minimum management skills (Jewell et al. 1979). Based on a simplified reactor design and reported advantages of lower capital and operational costs, the plug-flow digester has been the most readily utilized design compared with a completely mixed design for dairy manure. A variation of the plug-flow digester (especially when food wastes are added) is a horizontal mixed digester used to maintain solids in suspension. A new development is the design of a vertically mixed digester tank of either concrete or steel. The mixing is achieved by several methods, such as slow rotational paddles at several depths, submersible pumps or propellers, and side-entry circulating systems with the motive force outside the tank. Multiple tanks may be needed for larger farms and/or when food wastes and other organic materials are introduced as a co-digestion feedstock.

The U.S. EPA (2008) through its AgSTAR program estimated in December 2008 that there are 121 farm-scale digesters operating at commercial livestock farms in the United States (Table 4.2). Six of these installations are systems that provide manure treatment for multiple farms. In 108 of the 121 operational systems, the captured biogas is used to generate electrical power, with many of the farms recovering waste heat for the electricity generating equipment for on-farm use. The U.S. EPA estimates that these systems generate ~218,000 MWh of electricity per year. The remaining 13 systems burn the biogas in boilers, upgrade the gas for injection into the natural gas pipeline, or simply flare the captured gas.

Outlook

Anaerobic digestion of agricultural wastes is a mature technology with numerous full-scale digesters located all over the world. Noteworthy is the recent interest in digesting or co-digesting of bioenergy crops in the EU because of the relative high-energy efficiency of methanogenic food webs (no side product formation and product inhibition). Even though the level of maturity is high, research on reactor stability is necessary, especially when new applications are pursued. It is generally accepted by scientists and engineers that new problems and unanticipated challenges keep coming up. The complex microbial community and food web is the culprit, with more powerful techniques, such as metagenomics and stable-isotope probing, starting to shed light on the required mechanistic understanding of the microbial community and interactions (Lübken et al. 2007; Schlüter et al. 2008; Li et al. 2009).

For wastes from agriculture with complex nutrient and water cycles, anaerobic digestion should be seen in the larger context of an integrated system in which nutrients and water from digester effluent are continuously recycled. During the treatment of animal wastes with anaerobic digesters, for example, nutrients, such as ammonia and phosphate, are freed from biomass and are accumulated in solution. These nutrients must be recycled back to agricultural production in an environmentally friendly and sustainable way. In North Carolina (United States) an integrated system that recycled digester effluent to a greenhouse for tomato production (Cheng et al. 2004) was studied. Others are optimizing struvite precipitation to recover N and P (Borgerding 1972; Ohlinger et al. 1998; Schuiling and Andrade 1999; Battistoni et al. 2000; Münch and Barr 2001). In addition, upgrading the energy carrier methane to more valuable products may be necessary to guarantee economical viability. In Utah (United States) biogas from a swine waste digester was cleaned, steam reformed into synthesis gas (i.e., syngas), converted into methanol through a thermochemical process, and combined with triglyceride to produce biodiesel through a trans-esterification reaction process (Dugba 2003).

With the need for co-digestion, an opportunity exists to link agriculture, rural communities, and industry for sustainable rural community development. A U.S. example for this system approach is BioTown in Richmond, Indiana, where the goal is to create an energy self-sufficient community of about 500 persons using an anaerobic digester as an integrated technology to create biogas from animal manures, food wastes, organic municipal wastes, and crop residues (BioTown 2008). Communities such as these illustrate that anaerobic digestion is a significant and practical technology with relatively high-energy efficiencies and that agricultural wastes play an essential role.

Acknowledgements

LTA was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2004-35504-14896. In addition, The New York State Energy Research and Development Authority is gratefully acknowledged for partial support of anaerobic digestion studies at Cornell University. Finally, we thank Rodrigo Labatut and Nick Scalfone for generating Figures 4.1 and 4.2, respectively.

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Chapter 5

Conversion of Agricultural Residues to Bioethanol: The Roles of Cellulases and Cellulosomes

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Abstract

Some forms of agricultural residues represent an attractive resource for lignocellulosic biomass in our quest to reduce the dependence of the Western World on fossil fuels. After crops have been harvested, the residues usually represent relatively large amounts of cellulosic material that could be returned to the soil for its future enrichment in carbon and nutrients. However, today, many believe that a substantial portion of these residues could be made available for further conversion to biofuels. Likewise, animal wastes, particularly from herbivores and notably from ruminants, are high in cellulose content and can also be converted to liquid biofuels. Although such agricultural byproducts cannot compensate completely for the large volumes of liquid fuels required to sustain our transportation energy requirements, they can play a decisive local and regional role to fill these needs.

However, in this case, nature and mankind have different agendas. The challenge regarding cellulosic biomass is that cellulose plays a critical *structural* role in the terrestrial plant cell wall. Glucose, the most desirable plant sugar for fermentation, is incorporated within the cellulose microfibrils that make up the complex plant cell wall. The most successful future bioconversion processes for the production of biofuels from lignocellulose may indeed ultimately mimic the concerted action of the cellulolytic microbes, the bacteria, and fungi that have evolved to produce cellulases and cellulosomes. It is now very clear that the major bottleneck in this process—both from a biochemical and economical point of view—is the deconstruction of the plant cell wall, liberating both C6 and C5 sugars. Nature has evolved microbes and their enzymes to deal primarily with damaged and decaying vegetation. Ultimately, much of this plant matter is again converted to a form that can be incorporated into living plant tissue. Nature thus has the time needed to manage the plant biosphere with low-energy consuming processes that can be less than ideal. We, on the other hand, must deploy rapid, efficient, and most importantly, cost-effective conversion processes that will meet our future energy needs.

The present chapter deals with the current status of our knowledge regarding the function of cellulases and cellulosomes, and how we might use them in processes for biomass

conversion to biofuels. This includes a description of various types of cellulosic biomass in agricultural wastes and the pretreatment strategies required to enhance enzymatic attack and to avoid toxic byproducts that would interfere with enzyme action and fermentation. The effects of treatment with free cellulases versus treatment with cellulosomes are also detailed. The natural cellulases and cellulosomes, their various families, modular, and subunit architectures, are all documented. The search for novel enzymes, and strategies for mutation and modification of cellulases and cellulosomes for future application to bioenergy initiatives are considered as well. We address some of the bottlenecks and pitfalls that await us in our current and future efforts to provide efficient processes for conversion of cellulosic biomass to usable sugars for biofuel production.

Introduction

Lignocellulosic biomass has long been recognized as a potential low-cost renewable source of mixed sugars for fermentation to fuel ethanol (Lynd et al. 1991; Wheals et al. 1999; Lynd et al. 2002; Dien et al. 2003; Demain et al. 2005; Ragauskas et al. 2006; Schubert 2006; Himmel et al. 2007; Wall et al. 2008). One approach would be to degrade plant cell wall cellulose and hemicellulose to soluble sugars using severe chemistries, prior to conversion to ethanol, but economic and environmental issues preclude such strategies. The more accepted alternative is to employ microbial cellulases and related enzymes to cell walls that have been conditioned thermally and chemically in a milder process, known as “pretreatment.”

Several technologies have been developed over the past century that allow this conversion process to occur (Figure 5.1), yet the clear objective now is to make this process cost-competitive in today’s markets (Bayer et al. 2007). Perhaps the major bottleneck for

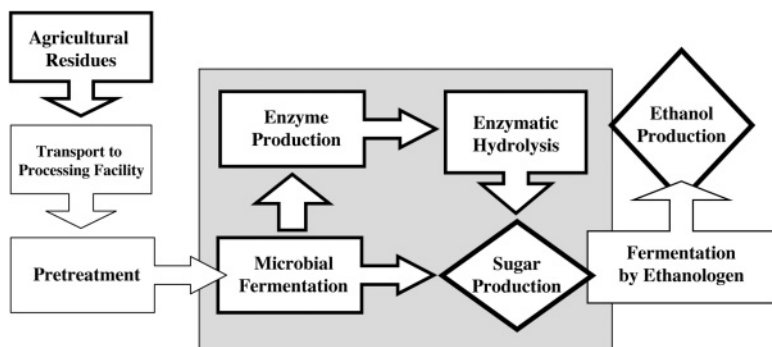


Figure 5.1. Major steps in conversion of plant cell wall biomass to biofuels. Following growth and harvesting of crops, the agricultural wastes are collected and transferred to a central processing facility. The pretreated plant cell wall material is used to grow cellulolytic fungal or bacterial cell cultures to produce large amounts of free sugars. Cellulolytic enzymes are also produced from the cells and are used to hydrolyze the pretreated biomass directly. Ethanogenic microbes (e.g., yeast or appropriate bacterium) are grown on the resultant sugars (glucose and other simple sugars), which results in the production of ethanol (or other fuels). The enzymatic breakdown of cellulose is the major bottleneck in the design of a cost-effective process.

conversion of biomass to ethanol is the high cost and low efficiency of the enzymes, the cellulases, and other glycoside hydrolases, which are capable of degrading crystalline cellulose and related plant cell wall polysaccharides. Efficient hydrolysis is impeded by limited accessibility of the enzymes and the recalcitrance of cellulose, owing to its extremely stable “microcrystalline” arrangement of the cellulose chains in the cell wall microfibrils.

The rate-limiting step in the hydrolysis of cellulose is not the catalytic cleavage of the β -1,4 bond, but the disruption of a single chain of the substrate from its native crystalline matrix, thereby rendering it accessible to the active site of the enzyme. Single cellulolytic enzymes alone are generally incapable of efficient cellulose hydrolysis. The mode of action of the various cellulases is different, and they are known now to act *synergistically*. Consequently, the secret to potent enzymatic degradation of the recalcitrant substrate is embedded in the knowledge of how these different types of enzymes *work together*.

This chapter describes the status of cellulases and cellulosomes *en route* to the efficient degradation of cellulosic biomass for the production of biofuels. We discuss the nature of cellulosic biomass in agricultural residues, various pretreatment strategies, and their effects on the microorganisms. We also discuss cellulolytic microorganisms, the various enzyme systems for biomass deconstruction, and future approaches for agricultural biomass deconstruction, while focusing on the production of soluble sugars. In doing so, we deem other topics as beyond the scope of the present chapter, notably microbial fermentation of soluble sugars to biofuels (Jeffries 2006; Hahn-Hägerdal et al. 2007a), metabolic engineering of bacteria, fungi, or yeast (Zhang et al. 1995; Hahn-Hägerdal et al. 2007b), and consolidated bioprocessing of cellulosic biomass directly to biofuels (Lynd et al. 2005; van Zyl et al. 2007; Lynd et al. 2008).

Plant Cell Wall Structure and Chemistry

The most abundant source of carbohydrates in the biosphere is plant biomass, which harbors the lignocellulosic materials comprising the cell walls of all higher plants. Plant cell walls are composed of polymeric networks. The predominant polymer and most abundant form of structural polysaccharide is cellulose. The second most abundant form of plant cell wall polysaccharide comprises the hemicelluloses and the third includes the pectins. The most abundant cell wall proteins are known as extensins and are classified as strongly basic glycoproteins. The non-polysaccharide aromatic polymer, lignin, is deposited after the cell has completed growing and is covalently cross-linked to hemicelluloses (Preston 1974). Plant cell walls are divided roughly into two types, the primary and the secondary cell walls.

The chemical composition and structure of primary and secondary cell walls are strikingly different. For example, cells that have only primary cell walls are often not lignified, such as in parenchyma tissue, whereas cells that have secondary cell walls may or may not be lignified, depending on their function in the plant tissue. Secondary cell walls are always more abundant than the primary cell walls in most plants and therefore represent the major reservoir of fermentable sugars in the plant.

Cellulose is a linear β -(1 $\rightarrow$ 4)-D-glucan and comprises 30%–50% of the plant cell wall. Pectins are “chelating-agent-extractable polysaccharides plus chemically similar inextractable polysaccharides” (Timell 1964, 1967). Pectins are rich in D-galacturonic acid and contain, in decreasing order, arabinose, galactose, and rhamnose. Hemicellulose is a general term used to refer to cell wall polysaccharides that are not celluloses or pectins. Hemicelluloses

include a variety of compounds, including xylans, xyloglucans, arabinoxylans, and mannans. Hemicelluloses almost always are branched with a wide spectrum of substituents along the backbone polysaccharide. Hemicelluloses in grasses and hard woods are primarily arabinoxylans, whereas those in soft woods are primarily galactoglucomannans (Aspinall 1959). Hemicelluloses are thought to undergo hydrogen bonding interactions with cellulose, as well as to other hemicelluloses, which form the basis for the microfibrillar structure of modern plant cell walls (Figure 5.2). The cell wall matrix is also established as a consequence of the esterification of hemicelluloses to lignins via *p*-coumaroyl and feruloyl groups (Mueller-Harvey et al. 1986). Hemicelluloses can, however, be extracted with alkali or dimethyl

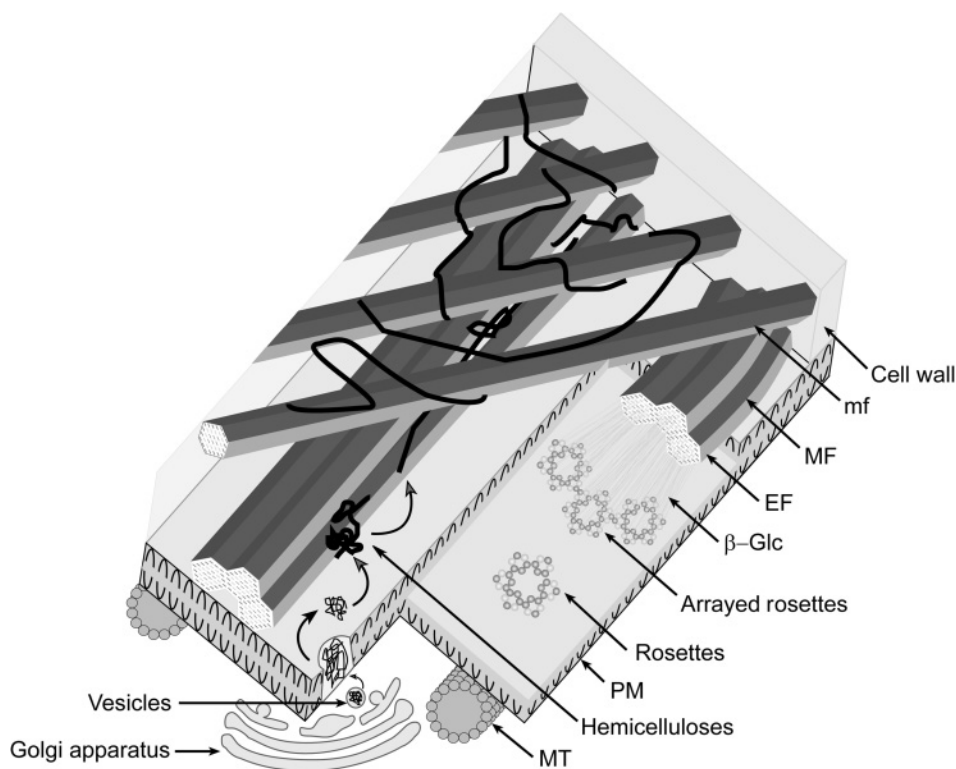


Figure 5.2. Model of the plant cell wall cellulose elementary fibril and its synthesis. In this model, at least three types of cellulose synthases (CesA subunits, a1, a2, and b) are needed to spontaneously assemble the rosettes that comprise 6×6 CesA enzymes synthesizing 36 β -1,4 glucan (β -Glc) chains forming the cellulose elementary fibril (EF). The rosettes may also form arrays in the cell membrane. In this case, a number of rosettes synthesize a bundle of the EFs, forming the microfibril (MF). The microfibril (mf) contains a single cellulose EF and hemicelluloses that are secreted from Golgi vesicles and coated on the EF surface. The estimated dimensions of EF are 3×5.5 nm. The depiction of the glucan chains is based generally on an X-ray structure of cellulose I β . It has been proposed that the 36-chain cellulose EF may contain three groups: 18 surface chains; 12 transition chains; and 6 core chains (Ding and Himmel 2006; Himmel et al. 2007). PM, plasma membrane; MT, microtubule.

sulfoxide, which disrupts the hydrogen bonds and, for alkali extraction, saponifies any ester cross-linkages to lignin (Bacon et al. 1975).

Pretreatment of Biomass

The major impediment to facile enzymatic degradation of plant cell wall biomass to biofuels is its inherent recalcitrance (Himmel 2008). The main contributions to biomass recalcitrance are the following:

- Epidermal tissue of the plant body, that is, cuticle and epicuticular waxes
- Arrangement and density of the vascular bundles
- Relative amount of sclerenchymatous (thick wall) tissue
- Degree of lignification
- Structural heterogeneity and complexity of cell wall constituents, that is, microfibrils and matrix polymers
- Challenges for enzymes acting on an insoluble substrate
- Inhibitors to subsequent fermentations that exist naturally in cell walls or are generated during conversion processes

For the above reasons, plant biomass has to be subjected to pretreatment processes in order to enable the enzymes to gain access to the cell wall polysaccharides, notably cellulose (Mosier et al. 2005; Wyman et al. 2005; Galbe and Zacchi 2007; Brunecky et al. 2009).

Current dilute acid biomass pretreatment processes hydrolyze cell wall hemicellulose in order to expose the cellulose fibers for simultaneous saccharification and fermentation (SSF). The resultant cellulose slurry is easily manipulated to the correct concentration in the bioreactor and can be enzymatically converted to free glucose. Acidic pretreatment liquor contains hemicellulose-derived sugars (monomers and oligomers), inhibitory compounds, and other soluble components and must be neutralized, concentrated, and possibly detoxified before microbial conversion. More severe biomass treatments that hydrolyze the hemicellulose and cellulose to free monomeric sugars, such as two-stage dilute or strong acid cooking, result in dilute sugar solutions that must be concentrated before conversion; as well as the uncontrolled production of sugar degradation products. In general, pretreatments that retain polymeric cellulose, such as a single-stage dilute acid or alkali pretreatments, and utilize conditions of moderate to low severity are preferred for subsequent microbial conversion (Brunecky et al. 2009).

Pretreatment Strategies

The three major methods of pretreatment that allow the recovery of solid cellulose are based on physical, biological, and chemical technology (Hsu et al. 1996).

- Physical pretreatments. Physical pretreatment involves reducing the size of the biomass particles so cellulases have access to the cell wall materials with reduced interference from the lignin and hemicellulose. An example of this is dry ball milling of biomass, which is very energy intensive and not likely to become practical at large scale (Zhu

et al. 2008). In physical pretreatment, most of the lignin, cellulose, and xylan remain in the solid phase.

- Biological pretreatments. Biological pretreatment uses enzymes or microorganisms to produce simple sugars from the carbohydrate polymers with minimal mechanical milling of the biomass. Although biological pretreatment is usually less energy intensive than chemical or mechanical pretreatment, it is still in the early stages of development (Sawada et al. 1995). Bio-pulping, not yet economically competitive with traditional pulping methods, is one example of biological pretreatment. Depending on the enzymes or microorganisms used, cellulose and/or hemicellulose may be hydrolyzed and even metabolized by the pretreatment strain.
- Chemical pretreatment. Chemical pretreatment is the most widespread method currently in use. Biomass is pretreated using acid or base, usually in combination with high heat and/or pressure. Many pretreatment conditions and catalysts have been used in biomass conversion, including concentrated and dilute acid, steam explosion, alkali, organic solvents, and ammonia. Biomass pretreatment has also been reviewed earlier by Dale (1985).

Acid hydrolysis is the most common method, with sulfuric, hydrochloric, and phosphoric acid all being used (Hsu et al. 1996). Nitric and peracetic acid have also been used, but their method of action is not polysaccharide hydrolysis, but rather by fiber matrix degradation through lignin oxidation. Acid hydrolysis is typically carried out under conditions that maximize hemicellulose hydrolysis and minimize cellulose degradation. The most widespread process is the use of dilute (less than 1% w/v) sulfuric acid in combination with heat and pressure. Dilute sulfuric acid is inexpensive and hydrolyzes the hemicellulose almost completely, while degrading little of the cellulose. The drawbacks of this method are the demand for corrosion-resistant equipment and disposal of large amounts of gypsum generated during neutralization. Sulfuric acid pretreatment leaches toxic metal ions from the equipment and converts small amounts of glucose to hydroxymethyl furfural and xylose to furfural. Other inhibitors produced include oxidized phenolics from lignin degradation and acetic acid from xylan hydrolysis (van Walsum et al. 1996). Phosphoric acid is a weaker acid, causes few waste disposal problems, and can be used as a nutrient by yeast after neutralization with ammonia. However, phosphoric acid is about eight times as expensive as sulfuric acid.

Pretreatment with lime at elevated temperatures has recently been developed as an alternative pretreatment. Chang and coworkers demonstrated that pretreating switchgrass with 0.1 g $\text{Ca(OH)}_2/\text{g}$ dry biomass at 100°C or 120°C for 2 hours removed 29% of the lignin while hydrolyzing only 10% of the cellulose and about 27% of the xylan (Chang et al. 1996). The xylan and cellulose contents of the original biomass were about 21% and 37% (w/w), respectively. Accounting for the loss of xylan in the pretreatment, the xylan hydrolysis was near 100% theoretical after treatment with commercial cellulase preparations. Xylan hydrolysis was probably enhanced through the alkaline deacetylation of the hemicellulose. Removing the ester-linked groups greatly enhances the digestibility of the xylan by exposing the xylan backbone to enzyme hydrolysis. Although lime pretreatment gave high yields and excellent digestibility, the sugar loss during the pretreatment process was significant, with 10% of the cellulose and 27% of the xylan lost to the liquid stream.

In addition to the already mentioned methods, other pretreatment methods have been used, including steam explosion, acid catalyzed steam explosion, ammonia fiber explosion, organic solvents, supercritical fluid, irradiation, oxidizing agents, alkali, liquid hot water, ammonia recycled percolation, and ammonia–hydrogen peroxide percolation (Iyer et al. 1996; Kim and Lee 1996).

Enzyme Components for Biomass Deconstruction

Cellulases

For the engineer seeking to improve upon the natural process of converting plant biomass to fermentable sugars, the key challenge is to make cell wall depolymerization a more rapid and less costly process. The cost of biomass ethanol production has been reduced dramatically over the past two decades, to the point where the fuel is now competitive for the blending market, but further processing cost-reduction opportunities have been identified that would make it competitive as a pure fuel without subsidies (Lynd et al. 1996). Because the cost of producing the enzymatic catalysts proposed in the SSF process is a critical issue, the available enzymatic activity must be maximized to effectively incorporate cellulases into these process schemes. This requirement can be met by ensuring that the enzymes used are obtainable at minimal cost and of the highest specific activity, the highest possible stability, and optimal in terms of pH and temperature tolerance.

While cellulosic biomass is produced at a rate of nearly 3×10^9 tons per year and represents 50% of all available biomaterial, the biologically mediated depolymerization of this resource has eluded clear, precise definition at the molecular level. Although the biological depolymerization of native plant matter requires a suite of glycoside hydrolases aided by chemical or mechanical conditioning, in many ways this problem is primarily one that focuses on the enzymes that act on cellulose. Many workers in the field agree that cellulose decrystallization and depolymerization are indeed the rate-limiting steps in biomass conversion (Himmel et al. 2007).

Hemicellulose removal by dilute acid treatment is a classical means of rendering biomass more amenable to cellulase action (Grohmann et al. 1985). Kong and coworkers (Kong et al. 1993) also showed that biomass with reduced acetylation responded significantly more favorably than native biomass to cellulase action. Biomass with reduced lignin content, or perhaps altered chemistry, appears to be more readily hydrolyzed by cellulases (Vinzant et al. 1997; Kristensen et al. 2007). The structural and reactive chemical features of the substrate (primarily defined as acetyl and lignin contents) can be pictured as controlling the accessibility of enzyme to cellulose; the degree of cellulose crystallinity can be visualized as controlling the hydrolytic rate (Jeoh et al. 2007).

The definitive enzymatic degradation of cellulose to glucose in fungi and most bacteria is generally accomplished by the synergistic action of three distinct classes of enzymes:

- The “endo-1,4- β -glucanases” or 1,4- β -D-glucan 4-glucanohydrolases (EC 3.2.1.4), which act randomly on soluble and insoluble 1,4- β -glucan substrates and are commonly measured by detecting the reducing groups released from carboxymethylcellulose (CMC). A relatively new subset of this class of cellulase, called “processive endoglucanases,” has recently been classified (Reverbel-Leroy et al. 1997; Wilson et al. 1998).
- The “exo-1,4- β -D-glucanases,” including both the 1,4- β -D-glucan glucosylhydrolases (EC 3.2.1.74), which liberate D-glucose from 1,4- β -D-glucans and hydrolyze D-cellobiose slowly, and 1,4- β -D-glucan cellobiohydrolase (EC 3.2.1.91), which liberates D-cellobiose from 1,4- β -glucans.
- The “ β -D-glucosidases” or β -D-glucoside glucosylhydrolases (EC 3.2.1.21), which act to release D-glucose units from cellobiose and soluble cellodextrins, as well as an array of glycosides.

Cross-synergism between endo- and exo-acting enzymes isolated from the same or different species, genera, or microbial families has been demonstrated many times (Wood and McCrae 1979; Coughlan et al. 1987; Eveleigh 1987). Exo–exo synergism was first reported in 1980 (Fägerstam and Pettersson 1980). It is currently believed that exo–endo synergism is explained best in terms of providing new sites of attack for the exoglucanases. The latter enzymes normally find available cellodextrin “ends” at the reducing and nonreducing termini of cellulose microfibrils. Random internal cleavage of surface cellulose chains by endoglucanases provides numerous additional sites for attack by cellobiohydrolases. Therefore, each hydrolytic event by an endoglucanase yields both a new reducing and a new nonreducing site. Thus, logical consideration of catalyst efficiency dictates the presence of exoglucanases specific for reducing termini and nonreducing termini. The principle of interspecies interchangeability of cellulase components is now the cornerstone of recombinant cellulase system design and construction. If indeed cellulase component enzymes are truly generalized in both structure and function, components can be selected and combined from a wide array of source organisms to form novel enzyme cocktails. For example, *Trichoderma reesei* cellobiohydrolase I (CBH I) has been shown to be a powerful element in multi-enzyme mixtures using either fungal or bacterial endoglucanases (Baker et al. 1998).

Xylanase/Cellulase Synergism

In the enzymatic hydrolysis of plant cell walls, cellulose digestion is highly dependent on hemicellulose digestion. Although other factors such as crystallinity and lignin content have been suggested as barriers to the enzymatic attack on lignocellulose (Kong et al. 1993), the key to increasing lignocellulose digestibility depends on the increase of the cellulose surface that is accessible to the enzymes. Hemicellulose surrounds the cellulose fibrils, protecting them from any biological attack. This makes it a necessity to hydrolyze hemicellulose first. It is now thought that digestion of hemicellulose loosens the rigid, complex structures covering the microfibrils of the cell wall and exposes the cellulose surface to cellulase attack (Ding and Himmel 2006). Indeed, recent studies of the augmentation of cellulase systems with xylanases and carbohydrate esterases demonstrate clearly that cellulose digestibility is linked to a synergistic relationship between these enzymes (Selig et al. 2008).

Families of the Glycoside Hydrolases

The cellulases and hemicellulases belong to a large group of enzymes called glycoside hydrolases, which hydrolyze the glycosidic bond between two or more carbohydrates or between a carbohydrate and a noncarbohydrate moiety. Previous classification schemes have been based usually on the substrate specificities of the enzyme, but such classification is largely inappropriate for the glycoside hydrolases, because single protein folds are known to harbor a diversity of substrate specificities. A better classification scheme has been instituted over a decade ago by Bernard Henrissat and colleagues (Coutinho and Henrissat 1999; Henrissat and Davies 2000; Cantarel et al. 2009), which is based on the amino acid sequence and consequent fold of the protein. The various glycoside hydrolases are thus divided into families, which currently number 114. This scheme serves to provide comparative structural features of the enzymes within a family, their evolutionary relationships, and their mechanism of action. A compendium of the glycoside hydrolases and related carbohydrate-active enzymes (CAZymes) can be found on the CAZY website (<http://www.cazy.org/>).

The members of most of the glycoside hydrolase families, relevant to this chapter, exhibit multiple types of activities on either cellulosic and/or hemicellulosic substrates—independent of the fold, although some of the families are restricted to a certain type of activity. The specificity of these enzymes is thus a function of the architecture of the active site, the carbohydrate binding modules(s), and the linker peptide(s); not necessarily dictated by the overall structure of the enzyme.

The enzymes of some families occur mainly or exclusively in fungi, for example, GH7, GH45, and GH61. Conversely, members of some other families occur mainly or exclusively in bacteria, for example, GH8, GH44, and GH48. The major glycoside hydrolases and their key substrate activities are listed in Table 5.1.

Indeed, the glycoside hydrolase is usually only part of the story, albeit the definitive “business” part of the protein where the actual bond cleavage of the target carbohydrate is performed. Nevertheless, hydrolysis is generally modulated by the action of additional ancillary components of the enzymes, usually in the form of modules—an independently folding sequence within the intact polypeptide. The glycoside hydrolase itself forms the major catalytic module of the enzyme. Various other modules may often be present. Some of these ancillary modules may have enzymatic activity, such as the carbohydrate esterases, notably the feruloyl and coumaroyl esterases and the acetyl xylan esterases, all of which are commonly found in association with xylanase catalytic modules. Moreover, some glycoside hydrolases comprise two or more catalytic modules (multiple GHs from one or more families). In view of the multi-modular nature of these enzymes, they sometimes reach extremely large molecular proportions.

The Carbohydrate-Binding Module (CBM)

The primary type of ancillary module, which is common to most glycoside hydrolases, is the CBM (Linder and Teeri 1997; Boraston et al. 2004). Many CBMs serve to target the parent glycoside hydrolase to the substrate. The first CBMs to have been described were initially termed CBDs (cellulose-binding domains), owing to their substrate specificities and binding to crystalline types of cellulose. The CBDs were thus divided into “types” on the basis of amino acid sequence, in a manner similar to the GH families. Further work, however, revealed that some of the CBD types were not specific for crystalline cellulose (such as type 4) or to cellulose at all (some members of type 2 bound to cellulose, whereas others bound to xylan). Moreover, some protein modules were found to exhibit binding specificity to non-cellulosic polysaccharides. Today, the different CBMs are now divided into over 50 different families showing broad specificity patterns, sometimes within a given family and even by a given module. The CBMs exhibit various functions, including targeting of the parent enzyme to the undigested substrate, targeting of given modules to portions (conformations) of the substrate during deconstruction, and attachment of the parent enzyme to the microbial surface.

Enzyme Systems

Cellulolytic bacteria and fungi produce a variety of different cellulases and related glycoside hydrolases, which together convert plant cell wall polysaccharides to simple fermentable sugars. Cellulolytic bacteria and fungi employ different strategies for the degradation of the

Table 5.1. Major glycoside hydrolase families and their enzymatic activities. The glycoside hydrolase families (GHn) in which some members exhibit standard *cellulase* activities are shown in bold. GH families that include cellulases exclusively are followed by an asterisk (*). See CAZy website for more details: <http://www.cazy.org/>.

GH Family	Enzymes
GH1	Numerous activities, including β -glucosidase, β -galactosidase, β -mannosidase, and β -glucuronidase; but <i>not</i> β -xylosidase activity
GH2	Numerous activities, including β -galactosidase, β -mannosidase, and β -glucuronidase; but <i>neither</i> β -glucosidase nor β -xylosidase activities
GH3	Numerous activities, notably not only β -glucosidase and β -xylosidase activities, but also glucan 1,3- β -glucosidase, glucan 1,4- β -glucosidase, and exo-1,3(4)-glucanase activities
GH5	Broad spectrum of cellulase and hemicellulase activities, including cellulase, xylanase, 1,3- β -mannanase; β -mannosidase, glucan 1,3- β -glucosidase, licheninase, glucan endo-1,6- β -glucosidase, mannan endo-1,4- β -mannosidase, endo-1,6- β -galactanase, and xyloglucan-specific endo-1,4- β -glucanase activities
GH6*	Cellulase activities in both aerobic bacteria and fungi (not found in archaea): both endo- and exo-glucanase (cellobiohydrolase) activities
GH7*	Cellulase activities <i>exclusive</i> to the fungi: both endo- and exo-glucanase (cellobiohydrolase) activities
GH8	Cellulase, lichenanase, xylanase activities; exclusive to bacteria
GH9*	Endo-, processive endo-, and exo-glucanase (cellobiohydrolase) activities in bacteria, plants, and fungi (but not in archaea)
GH10	Endo-1,4- β -xylanase and endo-1,3- β -xylanase activities in bacteria and fungi
GH11	Xylanase activities in bacteria and fungi
GH12	Endoglucanase, xyloglucanase, and 1,3(4)- β -glucanase in the three domains of life
GH16	Endo-1,3- β -glucanase, endo-1,3(4)- β -glucanase, lichenanase, and xyloglucanase activities
GH17	Glucan 1,3- β -glucosidase and lichenanase activities
GH18	Chitinases
GH19	Chitinases
GH26	β -Mannanase and 1,3- β -xylanase activities
GH30	1,6- β -Glucanase and β -xylosidase activities
GH39	β -Xylosidase activity
GH42	β -Galactosidase activity
GH43	Broad spectrum of hemicellulase activities, including xylanase, arabinanase, β -arabinofuranosidase, β -xylosidase, and galactan 1,3- β -galactosidase activities in bacteria and fungi
GH44	Endoglucanase and xyloglucanase activities, mainly in bacteria
GH45*	Endoglucanase activity, mainly in fungi (some bacteria)
GH47	α -Mannosidase activity, mainly in fungi
GH48*	Cellobiohydrolases and endo-processive cellulases; mainly in bacteria; an important enzyme in all cellulosomes and in some noncellulosomal systems
GH51	α -L-Arabinofuranosidase and endoglucanase activities
GH52	β -Xylosidase activity
GH53	Endo-1,4- β -galactanase activity
GH54	α -L-Arabinofuranosidase and β -xylosidase activities, mainly in fungi
GH55	Exo- and endo-1,3-glucanase activities, mainly in fungi
GH61	Exclusive to fungi. In some cases, annotated as endoglucanases, but probably disrupt cellulose structure rather than cleaving glucoside bonds.
GH62	α -L-Arabinofuranosidase activity
GH64	1,3- β -Glucanase activities; mainly in bacteria
GH67	α -Glucuronidase and xylan α -1,2-glucuronosidase activities
GH74	Xyloglucanase and endoglucanase activities
GH81	1,3- β -Glucanase activity

plant cell wall polysaccharide substrates, which reflect the complement and type(s) of enzymes produced by a given microbe. The resultant “cellulase system” may be characterized by free enzymes, cell-bound enzymes, multifunctional enzymes, cellulosomes, or any combination of the latter (Bayer et al. 2006; Wilson 2008).

Free Enzyme Systems

The free enzymes comprise a catalytic module alone with no accessory modules or with a CBM. The simplest enzymes often specialize on degrading soluble oligosaccharide breakdown products or bind to the polysaccharide substrate such as cellulose or xylan via the intrinsic affinity of its active site. In contrast, the polypeptide chain of many free enzymes includes both a catalytic module together with a CBM. This basic bi-modular arrangement can be further extended by the inclusion of additional types of modules or repeating units of the same module, all of which serve to modify the activity of the catalytic module on the polysaccharide substrate. The free enzyme, however, remains unattached to other enzymes and can work in an independent manner on a given substrate. The enzyme systems of aerobic fungi and bacteria usually contain numerous enzymes that are basically in the free state (Knowles et al. 1987; Wilson 1992; Warren 1993, 1996; Teeri 1997; Teeri et al. 1998; Wilson 2004; Viikari et al. 2007; Kumar et al. 2008).

Cell-Surface Enzyme Systems

In contrast to the free enzyme systems, some enzymes are attached directly to the cell wall. This is frequently accomplished in Gram-positive bacteria via a specialized type of module, the S-layer homology (SLH) module, previously shown to be associated with the cell surface of Gram-positive bacteria (Lupas et al. 1994). Attachment of enzymes to the cell wall may have evolved to provide a more efficient assimilation of the soluble sugars produced due to their proximity to the cell surface. This arrangement would serve to reduce competition with other bacteria for the soluble products.

Examples of putative cell surface enzymes that contain an SLH module include a GH5 cellulase and GH13 amylase-pullulanase from *Bacillus*, a GH10 xylanase from *Caldicellulosiruptor* (Saul et al. 1990), a GH5 endoglucanase from *Clostridium josui*, a GH16 lichenase and GH10 xylanase from *Clostridium thermocellum* (Jung et al. 1998), and a variety of enzymes (GH10 xylanases, a GH5 mannanase, and a GH13 amylase-pullulanase) from different species of *Thermoanaerobacter* (Matuschek et al. 1996). The modular architecture of these enzymes may be particularly intricate, containing numerous different modules in a single polypeptide chain, thus forming extremely large enzymes.

Multifunctional Enzyme Systems

Some cellulases exhibit a higher level of complexity whereby more than one catalytic module and/or CBM is included in the same protein. Examples of such enzymes are the very similar cellulases from *Anaerocellum thermophilum* (Zverlov et al. 1998) and *Caldocellum saccharolyticum* (Te'o et al. 1995), both of which contain a GH9 and a GH48 catalytic module. Other paired catalytic modules include those from GH44 and either GH5 or GH9. Such an arrangement presumably indicates close cooperation between two particular catalytic domains, which may lead to synergistic action on the cellulosic substrate.

Some xylanases also exhibit such a multi-modular structure. GH10 and GH11 xylanases may be linked in the same polypeptide chain either to each other, to GH5, GH16, and GH43 catalytic modules or to ferulic, coumaric, or xylan acetic acid esterases from different families of carbohydrate esterase. One particularly interesting combination of multifunctional catalytic modules that appear in the same polypeptide chain is a typical GH10 xylanase together with a CE1 feruloyl esterase, which presumably allows enhanced cleavage of the xylan–lignin linkage in the plant cell wall (Grépinet et al. 1988; Fontes et al. 1995).

It is striking to note that many of the multifunctional enzymes in nature occur in bacteria that inhabit extreme environments, for example, growing at 60°C–90°C. One exception is evident in the rumen bacterium, *Ruminococcus flavefaciens*, which produces some very intriguing multifunctional xylanases (Flint et al. 1993; Laurie et al. 1997). One could argue, however, that the rumen environment is indeed a very specialized environment (Flint et al. 2008). Why this bacterium “chooses” to produce such complicated enzymes remains a mystery.

Cellulosomes

Cellulosomes are multi-enzyme complexes that catalyze the efficient hydrolysis of cellulosic substrates and constitute a major paradigm of prokaryotic degradation of cellulose and related plant cell wall polysaccharides. The first cellulosome was discovered in the anaerobic thermophilic bacterium, *C. thermocellum* (Bayer et al. 1983; Lamed et al. 1983a,b). During the past 25 years or so, the cellulosome concept has been subject to numerous reviews that have chronicled its discovery, development, and potential (Lamed et al. 1983b; Lamed and Bayer 1988a,b, 1991, 1993; Felix and Ljungdahl 1993; Doi et al. 1994; Bayer et al. 1996, 1998, 2004, 2006, 2008b; Béguin and Lemaire 1996; Belaich et al. 1997; Karita et al. 1997; Shoham et al. 1999; Doi and Tamura 2001; Schwarz 2001; Doi and Kosugi 2004; Demain et al. 2005; Bayer and Lamed 2006).

In *C. thermocellum*, cellulosomes appear in both the cell-free and cell-bound forms, the latter being associated with polycellulosomal protuberance-like organelles on the cell surface (Bayer et al. 1985; Bayer and Lamed 1986). Later, cellulosomes were detected in other cellulolytic organisms (Lamed et al. 1987; Mayer et al. 1987), including *Acetivibrio cellulolyticus*, *Bacteroides cellulosolvens*, *C. acetobutylicum*, *C. cellulolyticum*, *C. cellulovorans*, *C. papyrosolvens*, and the two rumen bacteria: *Ruminococcus albus* and *R. flavefaciens* (Doi et al. 1994; Pohlschröder et al. 1994; Belaich et al. 1997; Ding et al. 1999, 2000, 2001; Morrison and Miron 2000; Ohara et al. 2000; Sabathe et al. 2002).

Cellulosome systems contain numerous structural and enzymatic components. A simplified schematic view of the *C. thermocellum* cellulosome and its interaction with cellulose is shown in Figure 5.3. The cellulosomal enzyme subunits are incorporated into the complex by means of a unique class of nonenzymatic, multi-modular polypeptide subunit, termed scaffoldin. The “primary” scaffoldins usually contain a family-3a CBM that provides the cellulose-binding function and multiple copies of a definitive type of cohesin module. On the other hand, the cellulosomal enzyme subunits carry a complementary type of module, called dockerin. The cohesin–dockerin interaction provides the definitive molecular mechanism that integrates the enzyme subunits into the cellulosome complex (Tokatlidis et al. 1991, 1993; Salamitou et al. 1994b). A second, divergent type of cohesin–dockerin interaction serves to attach the enzyme-laden scaffoldin to the cell surface by virtue of a second type of “anchoring” scaffoldin that contains one or more cohesins and an SLH module (Salamitou et al. 1994a; Lemaire et al. 1995; Leibovitz and Béguin 1996; Leibovitz et al. 1997).

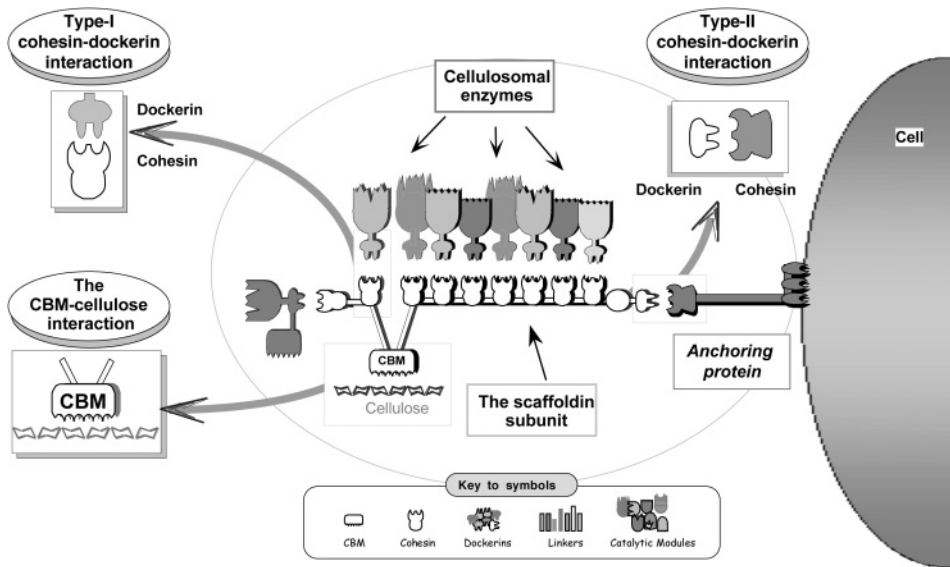


Figure 5.3. Simplified model of a typical cellulosome based on the *Clostridium thermocellum* cellulosome. The scaffoldin subunit comprises two main types of functional modules: a cellulose-binding module (CBM) and multiple copies of cohesins, which are interconnected by linker sequences. The CBM targets the cellulosome to its cellulose substrate. The catalytic subunits are integrated into the cellulosome complex by the mutual interaction between their resident type-I docking modules (dockerins) and the type-I cohesins of the scaffoldin subunit as shown in the insert. A second (type-II) cohesin–dockerin interaction attaches the cellulosome complex to the cell surface.

Until fairly recently, cohesins and dockerins were considered to be exclusive cellulosome “signature sequences”—that is, their presence is a good indication of a cellulosome in a given bacterium (Bayer et al. 1998). This perception has changed with the discovery of cohesins and dockerins, first in a non-cellulosomal archaeon (Bayer et al. 1999), and later in a broad number of non-cellulosomal archaea, bacteria, and primitive eukarya (Peer et al. 2009). The cohesins and dockerins are thus represented in all three domains of life, to the extent that their presence in the cellulosome might be the exception rather than the rule. Be that as it may, in the few known cellulolytic bacteria that produce cellulosomes, the cohesins and dockerins play a definitive role in cellulosome architecture and function.

The major difference between free and cellulosomal enzymes is that the free enzymes usually contain a CBM as an integral part of the polypeptide chain for guiding the catalytic module to the cellulosic substrate, whereas the cellulosomal enzymes bear a dockerin for their integration into the complex. Otherwise, both the free and cellulosomal enzymes can contain very similar types of catalytic modules. The cellulosomal enzymes collectively rely on the single scaffoldin-borne CBM3a for binding to the crystalline cellulose substrate.

In different cellulosomal bacteria, the modular architectures of the different scaffoldins, the location, and specificities of their various modular types—the cohesins, CBM, and/or dockerin—determine the overall composition and status of the cellulosome as a whole. Although the similarities among the different cellulosome species abound, their diversity and divergence from the *C. thermocellum* paradigm are striking. Each new cellulosome-producing

bacterium provides new and valuable information regarding the diversity of cellulosomes in nature.

The cellulosome was originally suggested to provide the anaerobic cellulolytic bacterium with distinct advantages in their efficient degradation of cellulosic substrates (Lamed et al. 1983a,b). The presence of the CBM on the common scaffoldin serves to deliver the enzymes *en block* to the substrate. Moreover, their close proximity on the scaffoldin subunit ensures their enhanced synergistic action. In addition to these advantages, the fact that the cellulosome is essentially a cell-surface organelle and the bacterial cell itself is attached to the substrate by virtue of the scaffoldin-borne CBM ensures that the cellulose-degradation products are preferentially available to the parent bacterium.

Future Approaches for Agricultural Biomass Deconstruction

The major bottleneck in the production of soluble sugars from cellulosic biomass involves the high cost of the enzymes necessary for this process. In theory, the solution should be simple: either to find cheaper ways of producing the enzymes or to find more active enzymes!

Actually, to improve the catalytic efficiency of cellulases may pose a formidable task, since these enzymes are, arguably, already of the most efficient in nature. Even if we were to improve the efficiency or thermal tolerance of one enzyme (acting alone), the question remains whether this improved enzyme will now work better in a synergistic mixture with the other enzymes. An exception to this consideration seems to be the case of the cellobiohydrolase I and II enzymes (GH7 and GH6, respectively) from fungi. In the free cellulose system, these enzymes are the star performers producing the majority of the soluble sugars from cellulose, aided primarily by only a small addition of endoglucanase activity.

Nevertheless, current strategies focus on the identification of new and improved enzymes, with the hope that those of highest activities will work best together. Since the improvements in producing industrial quantities of enzymes are more of a technical, engineering feat, we will concentrate here on strategies designed for improving enzyme activities or combinations thereof rather than improvements of processes for their production. In order to assemble improved enzyme systems for biomass conversion, a series of methodologies is required (Figure 5.4). New enzymes are identified by mining established enzyme databases, newly sequenced microbial genomes, and/or relevant cellulosic microenvironments. The newly identified enzymes are evaluated by genomic, proteomic, or metabolic profiling, and their activities are assessed. The properties of these enzymes can then presumably be improved by rational mutagenesis or directed evolution, leading to improved enzyme cocktails for enhanced conversion of cellulosic biomass to soluble sugars.

Search for Enzyme Diversity

To begin our search for new and diverse enzymes relevant to the deconstruction of the plant cell wall and conversion of their polysaccharides to soluble sugars, the best resource today is the CAZy database (<http://www.cazy.org/>). At this website, the various glycoside hydrolases and related CAZymes (notably the carbohydrate esterases and the pectate lyases) are categorized into families and listed with appropriate information and links. Tens of thousands of enzymes are catalogued, and vital details, both general and specific, are very easily accessible.

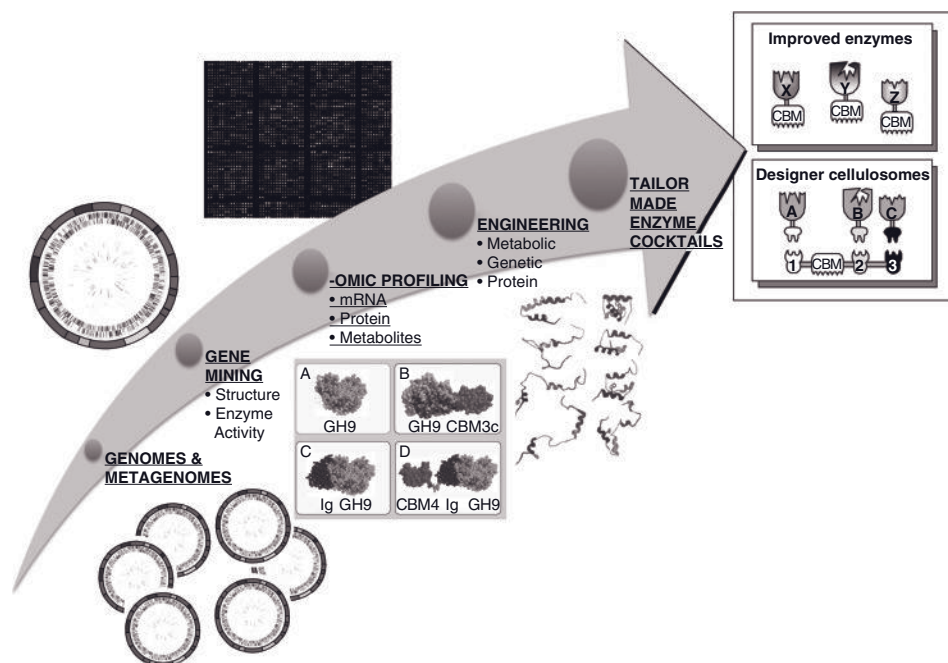


Figure 5.4. Continuum for discovery and optimization of carbohydrate-active enzymes.

In view of the fact that many aerobic fungal and bacterial free enzyme systems comprise only a few (six to ten) major endo- and exo-glucanases and similarly restricted numbers of hemicellulases, carbohydrate esterases, and pectate lyases, it is somewhat surprising that we could or would want to examine more than the known CAZymes for their applicability in conversion processes. How much more diversity do we need over and above the enzymes listed in the CAZy database? Surely, the natural enzyme systems, particularly those from a given microbial species, are most coordinated among themselves and have “learned through evolution” to interact in an optimized manner to achieve maximum degradation of the plant cell wall polysaccharide substrates.

Nevertheless, Mother Nature’s needs are different from ours, and the natural ecosystems are not necessarily attuned to the requirements of our human desires and our industrial processes designed to achieve them. Consequently, there is a current trend toward discovery of new enzymes and improvement of known enzymes for the purpose of conversion of large amounts of cellulosic biomass to their simple sugar constituents.

Genomic Approaches for Identification of Novel Glycoside Hydrolases

Lignocellulose-degrading microbes are found all over our planet, as free-living organisms and in the microbiomes of invertebrates and vertebrates. Genome sequencing of lignocellulose-degrading microbes is being used to reveal the relevant molecular components for optimal cellulose degradation in these microorganisms via sequence similarities to CAZyme components from other organisms. To date, there are at least 25 different microbes for which

genome projects are either in progress or completed (www.genomesonline.org/). Interestingly, based on the genome projects, there appears to be many different paradigms for lignocellulose-degradation, and each of these bacteria seem to have evolved organism-specific modes of plant cell wall deconstruction, which, while very efficient, are distinctly different.

This has led to comparative genome efforts, one of which is expression profiling. The idea is to monitor changes in gene expression in response to exposure to different plant cell wall substrates. This combined genomic and proteomic approach is needed to understand the regulation and assembly of this remarkable cadre of CAZyme and cellulosome components, and to identify those CAZymes that have maximal degradative capacity against a given substrate. To date, this approach has been used for two cellulosome-containing organisms, *Clostridium thermocellum* (Brown et al. 2007) and *Ruminococcus flavefaciens* (Berg et al. 2006). These functional and proteomic approaches can define candidate enzymes and are necessary for maximal lignocellulose degradation. Such functional and comparative genomics approaches are essential for defining how lignocellulose sources affect microbial-borne gene families and the rate and extent of lignocellulose degradation.

In the next few years, the powerful approach of comparative genomics will enable rapid advances. This is primarily due to the recent development of next-generation sequencing technologies, which have dramatically reduced the time, cost, and labor for genome sequencing projects. Pyrosequencing (also called 454 sequencing) was originally developed in the mid 1990s (Ronaghi et al. 1996, 1998) and has been continuously developed since then, and has become widely used in genome sequencing projects. The elimination of cloning vectors and their associated biases in terms of the clonability of certain DNA fragments is a major advantage in using this system (Hyman 1988; Ronaghi et al. 1996, 1998; Margulies et al. 2005). This sequencing technology also readily reads through secondary structures, and has the capacity to produce very large amounts of sequence. Current estimates from the latest version called “Titanium” suggest that read lengths with an average length of 400 bp and a five-fold throughput increase to 400–600 million bp per run for approximately \$12,000.

Other next generation of sequencing technologies also include the Solexa/Illumina 1G Genome Analysis System and Applied Biosystems SOLiD Sequencing. While presently average read lengths are much shorter than those obtained from the traditional methods, a far higher number of sequence reads can be produced in a single day or on a single run by these technologies. It is not unreasonable to predict that these next-generation technologies will eventually generate as good as or even longer read lengths than some of the traditional methods. Furthermore, there are additional “next-generation” technologies that will be released in the near term, including those from Helicos (www.helicosbio.com) and Complete Genomics (www.completegenomics.com).

These cost-effective next-generation sequence technologies will allow the generation of huge reference genome databases where one will now sequence up to 10 isolates of a microbial species for use in comparative studies. This approach was recently pioneered for microorganisms from the human gastrointestinal tract and their glycoside hydrolase components (Lozupone et al. 2008). Analysis of 67 microbial genomes from the human gastrointestinal revealed that the CAZyme repertoires found in these microbes had converged due to horizontal gene transfer, with limited evolution of the gene families. This implies that the environment can drive the adaptation of gene families. In this case, the plant cell wall material in the biome was the environment, and therefore the genes and enzymes needed for maximal degradation were the targets for optimization.

Metagenomics

Lignocellulose containing environments possess a natural degradative microbiome composed of a genomically diverse set of organisms. Often, these organisms, particularly those that are underrepresented, are missed in culture, but yet may supply significant metabolic contributions to surrounding organisms in these complex environments (Ley et al. 2005, 2008a,b; Sogin et al. 2006; Turnbaugh et al. 2006). Microbiologists began using culture-independent methods, metagenomics, to circumvent low isolation numbers (less than 1%) often seen with culture-based techniques in environmental samples (Handelsman 2004; Schloss 2008). Metagenomics allows genomic access to the entire population of microorganisms and allows for independent analysis of these microbes in conjunction with their natural habitat.

Traditional metagenomic analyses generally begin with total extracted genomic DNA of that community. The DNA can be digested using restriction enzymes, ligated into a vector and propagated in a host, often *Escherichia coli*. For a sequence-driven analysis, clones can be chosen at random and subsequently sequenced. For function-driven analyses, clones can be screened for phylogenetic markers, enzymatic activity, or antibody binding. Heterologous gene expression then allows for physiological identification of small molecules or proteins. Metagenomic studies began with traditional Sanger sequencing. As microscopic enumeration and colony counts were compared to the resulting numbers of microbes cultured, it became apparent that there was a large majority of organisms that were overlooked in these traditional studies (Schloss and Handelsman 2003; Handelsman 2004). Indeed, the sequencing and assembly of large gene insert libraries have also been hypothesized to lead to reconstruction of a nearly complete microbial genome (DeLong 2004). As demand for genomic tools arose that would allow for a more accurate picture of the functional distribution of the microbial diversity present, sequencing of large insert libraries, traditionally used in single organism genomics, was applied to total community DNA. This allows for the screening of clones for functional diversity resulting in novel gene discovery, providing a link for genetics and functional expression for each of the selected clones. Of particular significance to this review, metagenomic analysis of a bovine rumen expression identified 22 glycoside hydrolase clones of which four potentially represent previously undescribed families of glycoside hydrolases (Ferrer et al. 2005). A novel polyphenol oxidase (laccase) from this bovine rumen expression library has also been identified and characterized, and it was implied that this enzyme might play a role in ryegrass lignin digestion (Beloqui et al. 2006). Massive metagenome sequencing was also recently applied to another lignocellulose-degrading community, the termite hindgut (Warnecke et al. 2007). This extensive data set showed a diversity of bacterial genes for cellulose and xylan hydrolysis, mainly from spirochete and fibrobacter species. Clearly, the termite hindgut, like the rumen, is a microbial community specialized toward plant cell wall degradation and is a potentially important source of novel enzymes for more woody substrates.

With the advent of next-generation sequencing technologies, sequenced-based metagenomic approaches have strayed from cloning techniques, which introduce their own levels of bias, to a more random sequencing strategy, pyrosequencing (Ronaghi et al. 1996, 1998; Margulies et al. 2005). We have recently used this approach to examine randomly sampled pyrosequence data from three fiber-adherent rumen microbiomes and one pooled rumen liquid phase sample (Brulc et al. 2009). This genomic analysis revealed that, in the rumen microbiome, the dominant enzymes are those that attack the easily available side chains of complex plant polysaccharides and not the more recalcitrant main chains, even when cellulose is present as a substrate. Furthermore, when compared to the termite hindgut microbiome, there

are fundamental differences in the glycoside hydrolase content, with the termite hindgut microbiome containing more enzymes that are involved in degradation of cellulose (GH5, 9, 44, and 74) and xylan (GH10 and 11). Thus, it appears that in these lignocellulose-degrading microbiomes, CAZyme content appears to be diet driven (forages and legumes or wood). Therefore, when looking for novel microbial plant cell wall deconstructing enzymes, it is important to choose the environment that will serve as a genetic resource for plant cell-wall degrading microbial enzymes based on the substrates to be utilized.

Designer Cellulosomes

In recent years, the concept of designer cellulosomes has become a popular notion for a prospective solution to improving cellulase action, an approach first proposed over a decade ago (Bayer and Lamed 1992; Bayer et al. 1994; Ohmiya et al. 2003). But what is wrong with the native cellulosome systems? Indeed, they are considered to be the most efficient natural enzyme systems for cellulosic biomass, but the cellulosome-producing bacteria produce them in relatively small quantities, compared to the free cellulase systems produced by the aerobic fungi and bacteria. The current idea, therefore, would be to produce large quantities of artificial cellulosomes or cellulosomal components in an appropriate host cell system, which would then be cost-effective for industrial use.

Designer cellulosomes are artificial cellulosome complexes of defined composition, containing different recombinant enzyme components (Figure 5.5). The exact position of a given enzyme in the cellulosome can be predetermined by producing chimaeric scaffoldins that contain divergent cohesins of defined specificity and by attaching to the enzymes dockerins of matching specificity. Using this approach, the desired cellulosomes will self-assemble according to our initial design. In this manner, we can control the enzyme content

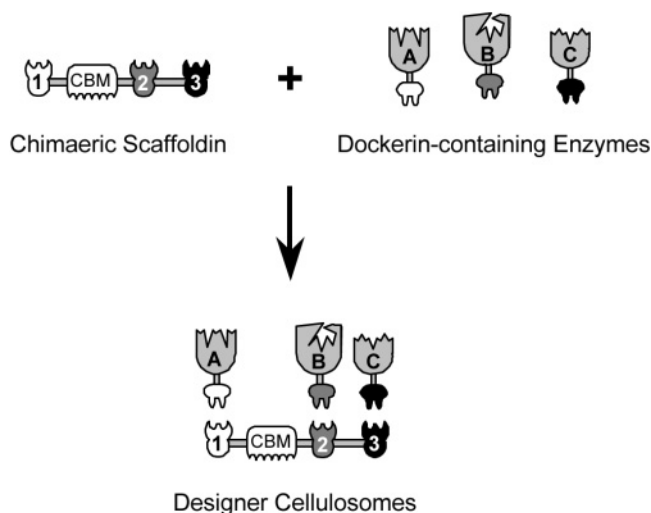


Figure 5.5. Construction of a designer cellulosome. A chimeric scaffoldin is designed containing a substrate-targeting CBM and multiple divergent cohesins (enumerated) for selective incorporation of the enzymes (A, B, and C). The recombinant enzyme hybrids contain a dockerin module selected for its matching specificity with one of the cohesins of the chimeric scaffoldin.

and position of the enzyme within the complex. Thus, any given complement of enzymes can be incorporated into such cellulosome complexes. These designer cellulosomes can be utilized as tools for both understanding cellulosome action and for future application in waste management (Bayer et al. 2007) and for production of biofuels (Bayer et al. 2008a,c).

The production of designer cellulosomes has been shown to be experimentally feasible. The first proof of concept involved the preparation of an exhaustive array of small bi-functional artificial cellulosomes, whose activity on specific substrates was examined (Fierobe et al. 2001, 2002). Small chimaeric scaffoldins were prepared that contained two divergent cohesins and matching dockerin-bearing cellulases, and their synergistic action on recalcitrant cellulosic substrates was demonstrated. Additional studies involved the action on crude native substrates, such as wheat straw, whereby designer cellulosomes containing a xylanase together with potent cellulases were demonstrated to dramatically enhance the degradation of complex lignocellulosic substrates (Fierobe et al. 2005). Later studies demonstrated that enzymes foreign to the native cellulosomes can also be included in the active state into designer cellulosomes (Caspi et al. 2006, 2008; Mingardon et al. 2007a), and radical designer cellulosome architectures can be produced at will, as long as the appropriate pre-design and experimental expertise are applied (Mingardon et al. 2007b).

To date, designer cellulosomes have been fabricated on a small scale using only two or three enzymes in complex and with only very modest gains in synergistic activity. Future work with designer cellulosomes should determine whether more impressive results can be obtained with larger numbers of enzymes within the individual designer cellulosomes, as we extend the size of designer cellulosomes to approach that of the native cellulosome systems. Another distinctive difference between the artificial and natural systems is that designer cellulosomes are uniform in composition, containing stoichiometric ratios of the desired enzymes whereas the native cellulosomes are heterogeneous in content and dispersion of its enzymes. The possible consequences of these differences vis-à-vis efficiency of deconstruction of cellulosic biomass are yet to be determined experimentally.

Mutagenesis of Cellulases and Other Glycoside Hydrolases

Once we have selected a set of enzymes to be included into a cellulase system—be it a free cellulase system, designer cellulosomes, native cellulosomes, or any combination thereof—it may be desirable to consider improving the enzymes. In theory, this can be accomplished by rational design or by directed evolution. Moreover, enzyme improvement can assume different forms. We may want to increase the activity of the enzymes. Alternatively, we may want to change the optima of their physical properties, such as temperature and pH. In changing the latter properties, directed evolution can be employed with some level of efficiency, since temperature and pH can be used as a selection pressure, and the stability of the mutated enzymes can be assayed relatively easily. Indeed, some success in the literature has been reported for such endeavors (Murashima et al. 2002; Wang et al. 2005; Choi et al. 2008). In recent studies (Hughes et al. 2006), combinatorial and robotic handling methods have recently been instituted for improvement of cellulase activity in individual endoglucanases. However, the methodology employed a soluble chromogenic substrate, and the approach was again taken with the intention of identifying mutants with heightened activities at low pH.

Improvement in cellulase activity *per se* is another story. It is one thing to isolate endoglucanase mutants using soluble substrates, but it is quite another to isolate mutants

of cellulases that work better on insoluble substrates. Moreover, the defining characteristic of cellulase and cellulosome action is *not* the improvement of a given cellulase, but how the different cellulases will work together to overcome the recalcitrant properties of the substrate. In this context, the rate-limiting step in the hydrolysis of crystalline cellulose is *not* the cleavage of the glycosidic bond of the cellulose chain, but the detachment of a single chain from the crystalline matrix (Bayer et al. 2007; Himmel et al. 2007, 2008a). Currently, there is really no acceptable assay for this function that can be employed for medium- or high-throughput procedures necessary for screening and selection of potent cellulases. Since there are no clear relationships between cellulase activities on soluble substrates and those on insoluble substrates, soluble substrates should not be used to screen or select for improved cellulases. Some exoglucanases, for example, show little or no activity on any substrate, but contribute substantially to the overall synergistic activity of enzyme mixtures and on insoluble cellulosic substrates. Theoretically, such assays should be based on relevant solid substrates, such as paper or plant cell walls (Zhang et al. 2006; Himmel et al. 2007). However, in practice, this has yet to be achieved in a reliable manner.

Conclusions

The structural polysaccharides in lignocellulosic biomass are a rich and renewable source of fermentable sugars for industrial production of biofuels. It is important to note that in attempting to utilize these carbohydrates at the commodity scale, we must overcome a key principle set forth in the evolutionary development of the cell wall of terrestrial plants: essential recalcitrance to deconstruction. Fortunately, progress is being made in this endeavor. Indeed, although these general process technologies are known, the key cost challenges remain the subject of considerable international research focus today. It is clear that only through dedicated, fundamental science guided by clearly defined applied objectives can such complex processes be made a reality. In this case, new and improved enzyme systems closely coupled to related process technologies, such as biomass pre-treatment, are required to provide cost-effective and large-scale quantities of liquid fuels from biomass.

Acknowledgments

The biomass structure, chemistry, and enzyme engineering review presented in this work was supported by the BioEnergy Science Center (a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science); the remainder of the review was supported the Israel Science Foundation (Grant Nos. 966/09 and 159/07), by grants from the United States–Israel Binational Science Foundation (BSF), Jerusalem, Israel, and by the National Research Initiative Competitive Grant Nos. 2002-35206-11634 and 2006-35206-16652 from the USDA Cooperative State Research, Education, and Extension Service. E.A.B. holds The Maynard I. and Elaine Wishner Chair of Bio-Organic Chemistry.

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Chapter 6

Fuel and Chemical Production from Glycerol, a Biodiesel Waste Product

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Abstract

Glycerol (or glycerin) is a byproduct of biodiesel, oleo-chemical, and bioethanol production processes. Due to the tremendous growth of the biofuel industry, glycerol is now regarded as a waste product, often with a disposal cost associated with it. Glycerol is abundant and inexpensive, and it is also a highly reduced molecule, which offers the opportunity to produce fuels and reduced chemicals at yields higher than those obtained using common sugars. Few microorganisms are able to utilize glycerol in the absence of external electron acceptors to produce high-value chemicals such as 1,3-propanediol, succinic acid, propionic acid, and biosurfactants. However, microorganisms that are amenable to industrial applications, such as *Escherichia coli* and *Saccharomyces cerevisiae*, were thought to metabolize glycerol only via respiration. We showed recently that *E. coli* can fermentatively metabolize glycerol, and we established pathways, mechanisms, and conditions of this metabolic process. Our findings have opened up a new platform for engineering *E. coli* for the production of several fuels and chemicals. This chapter will focus on the production of ethanol with coproducts hydrogen and formate from glycerol and highlight ways of improving yields and productivities of these products.

Renewable Sources of Glycerol

Glycerol is present in animal fats and oils in the form of triglycerides. These triglycerides consist of three fatty acids linked to three hydroxyl groups of glycerol through an ester bond. In most industrial applications, glycerol is recovered from the triglyceride molecules by breaking the ester bonds through various chemical or biological processes. Fatty acids present in triglycerides are directly converted into another material such as soap or biodiesel by saponification or transesterification, respectively (Figure 6.1; Yazdani and Gonzalez 2007). Glycerol is obtained from these processes as crude solution with various contaminants.

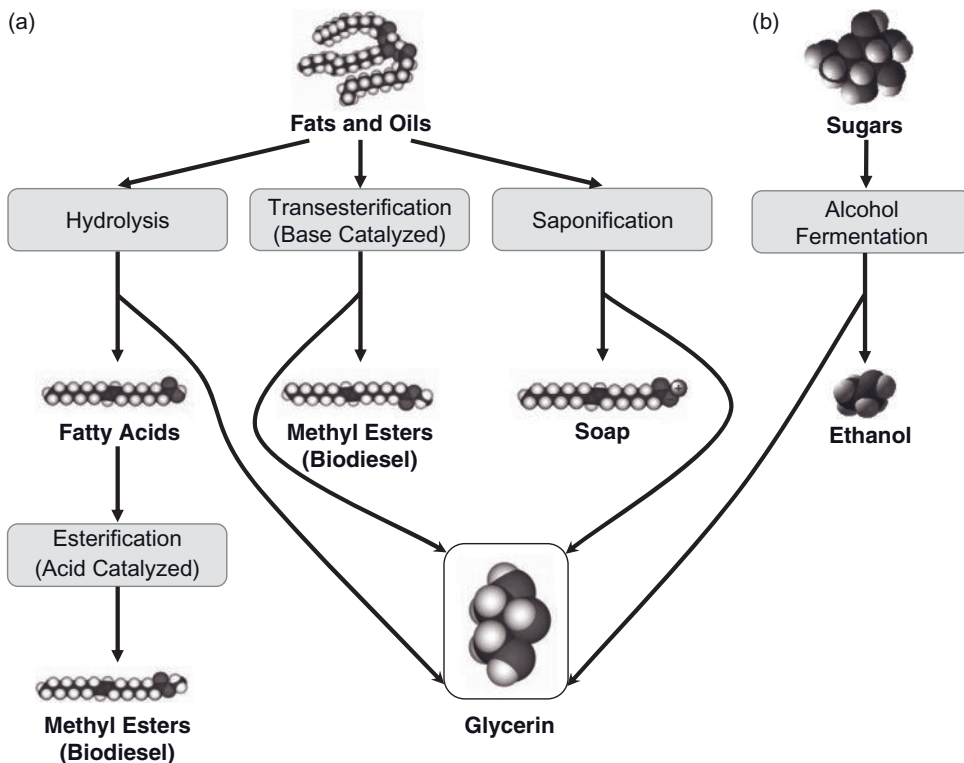


Figure 6.1. Two major platforms for the production of glycerol are described. The platforms are: (a) the biodiesel platform that utilizes fats and oils as starting material and (b) the bioethanol platform that utilizes sugar as starting material.

Biodiesel industries are the major sources of crude glycerol accumulation. During biodiesel production, fatty acid molecules are detached from the glycerol moiety of triglyceride by the action of a catalyst, and alcohol (methanol or ethanol) binds in its place through transesterification reaction (Figure 6.2). The catalysts commonly used for this purpose are sodium and potassium hydroxide. Reaction could take place at room temperature, albeit slowly. Heating triglyceride at 55°C helps completion of the reaction in 1–3 hours. The mixture is kept at room temperature for 1–2 days for the separation of glycerol and biodiesel to occur. Glycerol is heavier than ester and settles at the bottom of the container (Figure 6.3). For every 100 lb of biodiesel produced by the transesterification of triglycerides, 10 lb of crude glycerol is generated.

Pure glycerol is primarily used in the production of various foods, beverages, pharmaceuticals, cosmetics, and so on. The cost of purifying crude glycerol is high and uneconomical. Pure glycerol is currently sold at a relatively high price, which is in the range of \$0.60–\$0.90/lb. The high cost associated with the purification of crude glycerol makes it unattractive for biodiesel companies to invest in the purification process. The number of companies producing biodiesel has grown since the early 1990s due to increased demand for biodiesel fuel. This has led to a huge glut of crude glycerol. Crude glycerol used to be a desired coproduct that

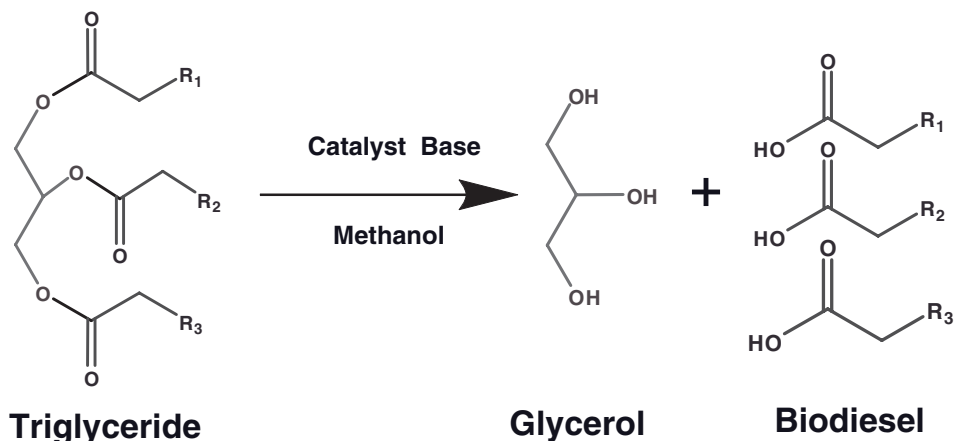


Figure 6.2. The production of biodiesel. The alcohol reacts with the fatty acid component of triglyceride (fat or oil) in the presence of a catalyst such as NaOH, KOH, H_2SO_4 , and so on to form alkyl ester (biodiesel) and glycerol.

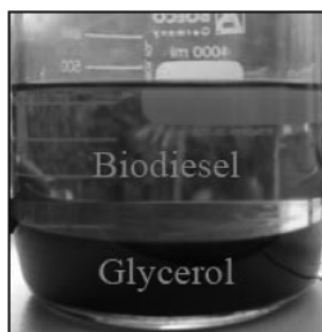


Figure 6.3. Biodiesel stored in a bottle upon completion of transesterification reaction. Glycerol is heavier than biodiesel and settles at the bottom of the flask.

contributes to the economics of biodiesel production but has now become an unwanted waste stream with disposal cost associated with it.

Companies that produce bioethanol are another well-known source of crude glycerol accumulation (Figure 6.1). During sugar fermentation to ethanol, yeast produces a substantial amount of glycerol in response to external osmotic stress (Figure 6.4). In a conventional fermentation, 4 g of glycerol is generated for every 48 g of ethanol produced and 100 g of reducing sugar consumed (<http://www.freepatentonline.com/5177008.html>). There are more than 170 ethanol production facilities in the United States with a total annual capacity of more than 10 billion gallons of ethanol (<http://www.ethanol.org/index.php?id=77>), resulting in several million gallons of crude glycerol production annually.

The surplus glycerol generated by the biodiesel and bioethanol industries will not only reduce the price of crude glycerol, but its disposal will also become a major issue. The

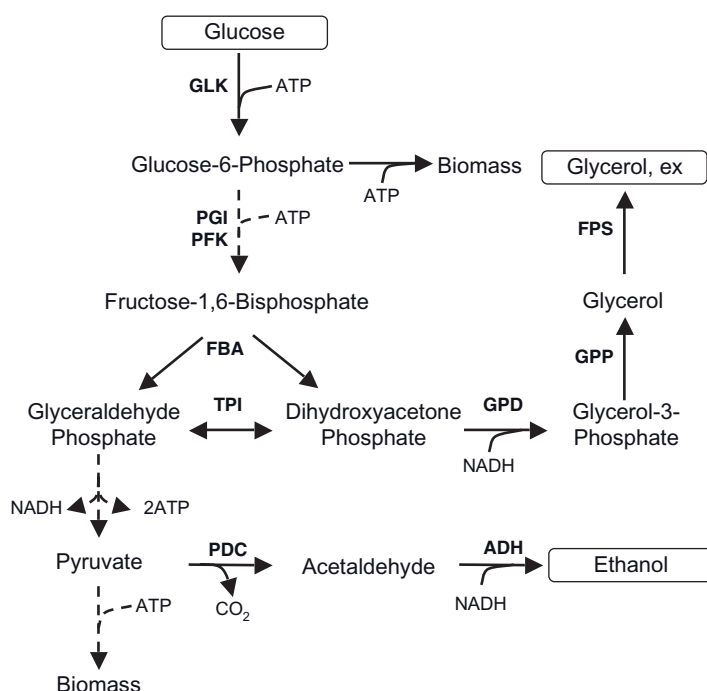


Figure 6.4. Glycerol production pathway in *Saccharomyces cerevisiae* during fermentation of sugar under osmotic stress. Relevant enzymes in the pathway are shown. Broken arrows represent more than one step. GLK, glucokinase; PGI, phosphoglucoisomerase; PFK, phosphofructokinase; FBA, fructose-1,6-bisphosphate aldolase; TPI, triose phosphate isomerase; GPD, glycerol-3-phosphate dehydrogenase; GPP, glycerol-3-phosphate phosphatase; FPS, glycerol transporter; PDC, pyruvate decarboxylase; ADH, alcohol dehydrogenase.

development of processes that can convert crude glycerol into high-value products is expected to make biofuels economically viable.

Conversion of Glycerol into Higher Value Products

Glycerol can be converted into higher value products by either biological or chemical transformations. Biological conversions are generally preferred to chemical conversions because biological conversions have higher reaction specificities, lower reaction temperatures and pressure, and fewer chemical contaminants than chemical conversions. Crude glycerol generated via biotransformations can be used as a carbon source for other microbial fermentations. The carbon atoms in glycerol molecules are highly reduced, and the conversion of glycerol to glycolytic intermediates generates twice the amount of reducing equivalents generated by glucose or xylose metabolism. For example, conversion of 1 mol of glycerol (a three-carbon molecule) to phosphoenol pyruvate (PEP) or pyruvate generates 2 mol of NADH, while conversion of 0.5 mol of glucose (a six-carbon molecule) or 0.6 mol of xylose (a five-carbon molecule) to PEP or pyruvate generates only 1 mol of NADH. As a result, yields of fuels and chemicals are higher when synthesized from glycerol than monosaccharides.

Uptake of glycerol across the cytoplasmic membrane by cells can occur via facilitated diffusion and active transport. In *E. coli*, the uptake of glycerol is through facilitated diffusion, mediated by an integral membrane protein, the glycerol facilitator GlpF (Heller et al. 1980; Voegelé et al. 1993). This intracellular glycerol is phosphorylated by the glycerol kinase and the glycerol-3-phosphate formed remains trapped inside the cell. In *S. cerevisiae*, the uptake of glycerol is mediated by either facilitated diffusion or active transport (Wang et al. 2001).

Although glycerol is commonly used by many microorganisms under aerobic condition, the highly reduced nature of glycerol can be exploited for the production of numerous bio-products. Few members of the *Enterobacteriaceae* family such as *Citrobacter freundii* (Homann et al. 1990) and *Klebsiella pneumoniae* (Forage and Foster 1982; Biebl et al. 1998) have been reported to ferment glycerol in a 1,3-propanediol (1,3-PDO)-dependent manner. Species such as *Clostridium pasteurianum* (Luers et al. 1997; Biebl 2001), *Clostridium butyricum* (Biebl 1991), *Enterobacter agglomerans* (Barbirato et al. 1996), *Enterobacter aerogenes* (Ito et al. 2005), and *Lactobacillus reuteri* (Talarico et al. 1990) have also been reported to ferment glycerol. A brief summary of the fermentation products of crude glycerol is given in Figure 6.5.

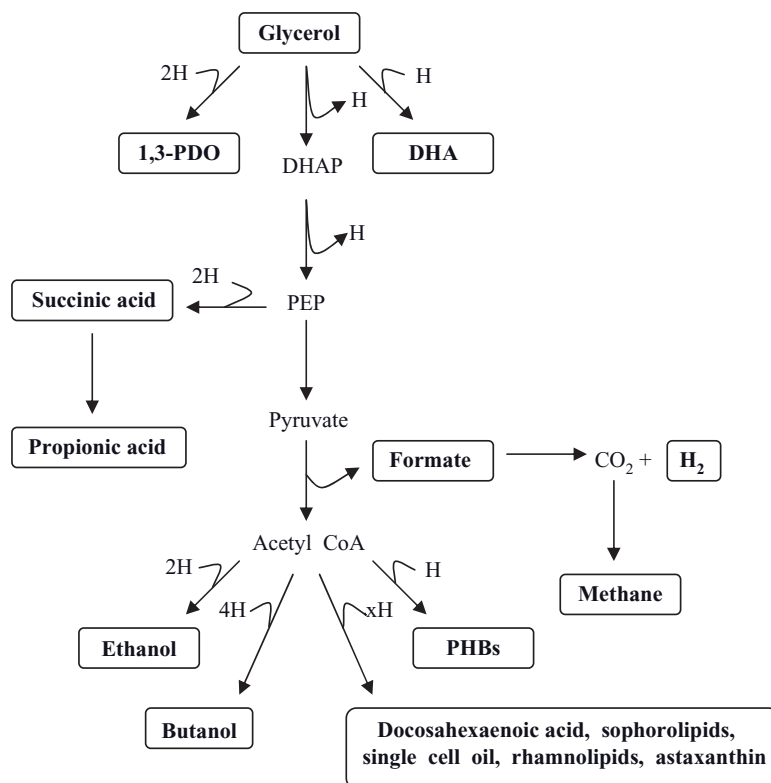


Figure 6.5. A diagrammatic representation of products of glycerol fermentation. H, NADH; 1,3-PDO, 1,3-propanediol; DHA, dihydroxyacetone; DHAP, dihydroxyacetonephosphate; PEP, phosphoenolpyruvate; PHBs, polyhydroxybutyrate.

1,3-PDO

1,3-Propanediol is a valuable feedstock chemical that is commonly used as a monomer for the synthesis of polyethers, polyesters, and polyurethanes. It can also be used for the production of cosmetics, lubricants, foods, medicines, composites, adhesives, laminates, powder and UV-cured coatings, moldings, and anti-freeze. Glycerol is metabolized via oxidative and reductive pathways in *Klebsiella*, *Citrobacter*, *Clostridium*, and *Enterobacter* species. In the oxidative pathway, the nicotinamide adenine dinucleotide (NAD)-dependent glycerol dehydrogenase (GldA) catalyzes the conversion of glycerol to dihydroxyacetone (DHA) with subsequent phosphorylation by dihydroxyacetone phosphate (DHAP) kinase to produce DHAP (Daniel et al. 1995) after which DHAP enters the glycolytic pathway. The reductive pathway is catalyzed by coenzyme B12-dependent glycerol dehydratase, which converts glycerol to 3-hydroxypropionaldehyde (3-HPA). 3-HPA is reduced to 1,3-PDO by NADH-dependent enzyme 1,3-propanediol dehydrogenase (1,3-PDODH). As the conversion of glycerol to 1,3-PDO results in the net consumption of reducing equivalents, this pathway provides a mechanism for achieving redox balance in the absence of electron acceptors such as oxygen, nitrate, and fumarate.

Glycerol fermentation by *Klebsiella* results in the accumulation of two main products, 1,3-PDO and acetate, whereas *Clostridium* species produce butyrate and 1,3-PDO. Although high levels of 1,3-PDO are produced by *Klebsiella* species, their use on an industrial scale is limited because they are opportunistic pathogens capable of causing urinary tract and abdominal infections, and pneumonia. Therefore, *Clostridia* are preferred because many *Clostridium* species of biotechnological importance are not pathogenic and certain species do not require the presence of vitamin B12.

When batch fermentations with *C. butyricum* were performed using crude glycerol at a concentration of 112 g/L, the maximum amount of 1,3-PDO obtained was about 63.4 g/L with a specific yield of 0.69 mol/mol glycerol (Table 6.1; Barbirato et al. 1998). A newly isolated strain of *C. butyricum* was found to produce up to 46 g/L of 1,3-PDO with a high volumetric productivity of 3.4 g/L/h (Papanikolaou et al. 2000). In another report, continuous fermentation with *K. pneumoniae* yielded 35.2–48.5 g/L of 1,3-PDO. In that report, a low concentration of glycerol was used initially and the concentration was gradually increased to prevent accumulation of substrate in the bioreactor (Menzel et al. 1997). The final volumetric productivity was in the range of 4.9–8.8 g/L/h. The addition of fumarate to the cultures of *K. pneumoniae* enhanced the utilization of glycerol and led to increased 1,3-PDO formation. Although the net 1,3-PDO yield of 0.57 mol/mol glycerol was obtained with or without the addition of fumarate, the volumetric productivity increased up to 17 mmol/L/h, an increase of almost 36% when compared to the corresponding control where glucose was used as substrate (Lin et al. 2005). This increase was attributed to two main factors. First, the addition of fumarate increased the activities of major enzymes involved in glycerol utilization and 1,3-PDO synthesis, that is, GldA, glycerol dehydratase, and 1,3-PDODH, which increased the metabolic flux toward 1,3-PDO production. Second, higher levels of fumarate led to a decrease in the NAD⁺/NADH ratio, which resulted in a greater concentration of reducing equivalents and hence a greater conversion of 3-HPA to 1,3-PDO.

Succinic Acid

Succinic acid is an important chemical used as an additive in food and pharmaceutical products, surfactants, detergents, solvents, and biodegradable plastics. Succinic acid can

Table 6.1. High-value products synthesized from glycerol via microbial fermentation.

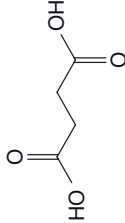
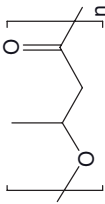
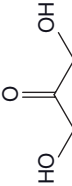
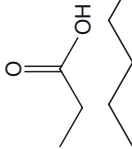
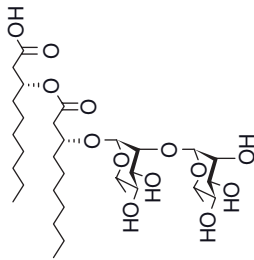
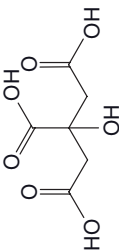
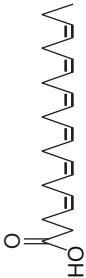
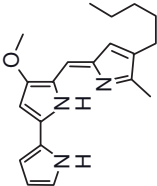
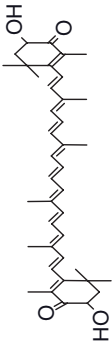
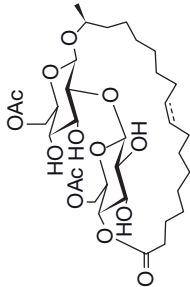
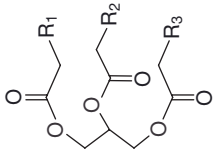
Product	Structure	Organism	Concentration (g/L)	Yield (mol/mol)	References
1,3-Propanediol		<i>Clostridium butyricum</i> <i>Clostridium butyricum</i> (Engineered)	63 31–48	0.69 0.66	Barbato 1998 Papanikolaou 2000
Succinic acid		<i>Klebsiella pneumoniae</i> <i>Anaerobiospirillum succiniproducens</i> <i>Actinobacillus succinogenes</i> <i>Mannheimia</i> sp	35–48 19 4.9 8.4	NA NA 1.0 0.93	Menzel 1997 Lee 2004 Lee 2001 Scholten 2008
Bio-ethanol		<i>Enterobacter aerogenes</i> <i>Escherichia coli</i> <i>Escherichia coli</i> (Engineered)	3.2 3.0 10	0.56–1.0 0.86 1.02	Ito 2005 Dharmadi 2006 Shams Yazdani 2008
Bio-hydrogen	H ₂	<i>Enterobacter aerogenes</i> <i>Escherichia coli</i> (Engineered)	0.12 0.45	0.85 0.96	Nishio and Nakashimada 2007 Shams Yazdani 2008
Bio-methane	CH ₃	<i>Methanogens</i>	NA	232 ^a	Khanal 2008
Polyhydroxy-butyrate		<i>Escherichia coli</i> (Engineered) <i>Methylobacterium rhodesianum</i> MJ126-J <i>Ralstonia eutropha</i> DSM11348	11 50 50	NA NA NA	Nikel 2008 Bormann 1999 Bormann 1999
Dihydroxy-acetone		<i>Gluconobacter oxydans</i> (Engineered)	30	0.6	Gatgens 2007
Propionic Acid		<i>Propionibacterium acidipropionici</i>	42	0.84	Barbato 1997
Butanol		<i>Clostridium pasteurianum</i>	17	NA	Biebl 2001
Rhamnolipids		<i>Pseudomonas aeruginosa</i>	15	NA	Zhang 2005

Table 6.1. Continued.

Product	Structure	Organism	Concentration (g/L)	Yield (mol/mol)	References
Citric Acid		<i>Yarrowia lipolytica</i>	35	0.20	Papanikolaou 2002
Docosahexa-enoic Acid		<i>Schizochytrium limacinum</i>	5	NA	Chi 2007
Prodigiosin (pigment)		<i>Serratia marcescens</i>	0.58	NA	Tao 2005
Astaxanthin (pigment)		<i>Phaffia rhodozyma</i>	0.034	NA	Kusdiyantini 1998
Sophorolipid		<i>Candida bombicola</i>	60	NA	Ashby 2005
Single Cell Oil		<i>Yarrowia lipolytica</i>	8.1	0.43	Papanikolaou 2002

^aValue in mL/g.
NA, not available.

also be used for the synthesis of 1,4-butanediol, tetrahydrofuran, γ -butyrolactone, adipic acid, *n*-methylpyrrolidone, and linear aliphatic esters (Zeikus et al. 1999). Succinic acid is generally produced under anaerobic conditions through carboxylation of phosphoenol pyruvate, a glycolytic intermediate. The carboxylation of PEP can occur by two mechanisms, either by phosphoenolpyruvate carboxylase (PPC) or by phosphoenolpyruvate carboxykinase (PEPCK). Conversion by PEPCK is energetically favored because an ATP molecule is produced during the reaction, thus regenerating the ATP used up during glycolysis, while in the case of PPC, this energy is dissipated via release of inorganic phosphate molecules. *Anaerobiospirillum succiniciproducens* is one of the most efficient succinate producers. It uses the PEP carboxylation pathway catalyzed by PEP carboxykinase (or PEP carboxylase), malate dehydrogenase, fumarase, and fumarate dehydrogenase (Lee et al. 2004). This microbe produced 19 g/L of succinate when glycerol was used as the sole carbon source in the culture medium (Table 6.1). *Actinobacillus succinogenes*, another natural producer of succinate, was found to produce 4.9 g/L of succinate with a specific yield of 1.3 g/g glycerol (Lee et al. 2001). This organism uses PEPCK, an energy-efficient enzyme, to convert PEP to oxaloacetate in contrast to *E. coli* and many other microbes that use PPC. Overproducing *A. succinogenes* PEPCK enzyme in *E. coli* strain improved succinate production in media containing 120 mM NaHCO₃ (Kim et al. 2004). In another study, succinate production was evaluated in strains overproducing PPC, and it was found that the level of succinate was enhanced by the simultaneous overexpression of PPC and pantothenate kinase (Lin et al. 2004). The overexpression resulted in an increased level of acetyl coenzyme A (acetyl-CoA), an activator of PPC (Lin et al. 2004). A novel succinic acid-producing bacterial strain designated as DD1 was isolated from the rumen of a cannulated Holstein cow (Scholten and Dagele 2008). This facultative anaerobe, which belongs to the family *Pasteurellaceae* and resembles the genus *Mannheimia*, produced 5.8 g/L succinic acid from glucose or sucrose with a yield of 0.6 g/g and a productivity of 1.5 g/L/h. When crude glycerol was used as the carbon source in continuous fermentations, the amount of succinic acid produced was 8.4 g/L; the yield was 1.2 g/g glycerol; and the productivity was 0.9 g/L/h. The high yield of succinic acid obtained from the fermentation was attributed to the highly reduced nature of carbons in glycerol molecules.

Hydrogen

Hydrogen is produced during fermentative metabolism of glycerol via conversion of pyruvate to formate, a reaction catalyzed by the enzyme pyruvate formate lyase (PFL). The formate is converted to carbon dioxide and hydrogen by the action of formate hydrogen lyase (FHL). This is probably essential for maintaining both the CO₂ supply needed for cell growth (Dharmadi et al. 2006) and the production of a proton motive force (PMF) that is essential for cell viability (Bagramyan and Trchounian 2003; Hakobyan et al. 2005). Under certain environmental conditions, engineered *E. coli* was found to produce hydrogen at the rate of 4.64 mmol/L/h with a specific yield of 0.96 mol H₂/mol glycerol (Yazdani and Gonzalez 2008). Biohydrogen production from glycerol containing waste generated from a biodiesel plant was studied using a natural isolate *E. aerogenes*. The rate of hydrogen production was determined to be 63 mmol/L/h with a specific yield of 0.85 mol H₂/mol glycerol (Ito et al. 2005; Nishio and Nakashimada 2007). Production of biohydrogen by *E. aerogenes* using crude glycerol as substrate affords biodiesel industries the opportunity to add value to crude glycerol and potentially increase their revenue base.

Methane

Methane is generally produced by methanogens, a group of over 50 different microorganisms belonging to Euryarchaeota. These microorganisms produce methane by the reduction of either CO₂ or acetate, while some have the ability to use methylated compounds such as methanol and methylamine for methane production. Biomethane produced from waste from biodiesel and other biofuel plants can be used to generate energy for their operation. This may dramatically reduce the dependence of the biofuel plant on external energy inputs such as natural gas and coal. However, direct utilization of crude glycerol streams by microbes is not efficient because of high salt levels associated with biodiesel waste streams (glycerol). This problem may be overcome either by diluting the waste stream or by mixing it with other waste streams. Theoretically, 0.43 L methane is produced for every gram of pure glycerol. This yield will obviously go down as the glycerol content of the waste stream decreases. When crude glycerol from swine manure (1.2 g glycerol/g swine manure) was used as the sole carbon source, methane was produced at a yield of 232 mL/g glycerol. However, when the glycerol/swine manure ratio was 4.6 or higher, the methane production was negligible, indicating that high glycerol levels are toxic to methanogens mainly due to high salt and methanol levels (Khanal 2008).

Polyhydroxyalcanoates

Polyhydroxyalcanoates (PHA) are a class of naturally occurring polyesters that are synthesized by various microorganisms as a reserve material for carbon and energy. When the bacteria are grown under low nutrition conditions, PHAs accumulate in the cytoplasm of the bacteria as hydrophobic granules and act as a carbon reservoir as well as an electron sink. Since these polymers are biodegradable and also biocompatible, they are of high demand in the pharmaceutical, fiber, and horticulture industries. In *Ralstonia eutropha* and most other PHA accumulating bacteria, polyhydroxybutyrate (PHB) is synthesized from acetyl-CoA through three enzymes: (1) β -ketothiolase, which condenses two molecules of acetyl-CoA to β -acetoacetyl-CoA, (2) an NADPH-dependent acetoacetyl-CoA reductase that catalyzes the formation of D-(2)-3-hydroxybutyryl-CoA (3HB-CoA), and (3) PHA synthase, which polymerizes 3HB-CoA to PHB (Schubert et al. 1988; Slater et al. 1988). Currently, industrial synthesis of PHAs involves aerobic processes that are energy intensive. There is a need, therefore, to develop newer fermentative ways of synthesizing these compounds. When *Methylobacterium rhodesianum* MJ 126-J and *R. eutropha* DSM11348 were cultured in a medium containing crude glycerol and casein hydrolysates, the amount of PHB produced was 50 g/L and the net conversion of glycerol to PHB was 17% (Bormann and Roth 1999). *E. coli* has also been engineered to produce PHBs by mutating the *arcA2* locus. The ArcAB system of *E. coli* represses genes that encode the enzymes involved in aerobic respiration, such as those of the tricarboxylic acid cycle, under anaerobic and microaerobic conditions (Salmon et al. 2005). The mutation in *arcA* region would elevate the tricarboxylic acid cycle activity, which supplies large amounts of reducing equivalents such as NADH and NADPH. Excess reducing equivalents favor the formation of an electron sink like PHBs (Nikel et al. 2006). The recombinant *E. coli* with mutation in *arcA* produced 1.44 g/L of PHBs in glucose media while the corresponding non-mutant produced only 0.07 g/L of PHBs. When recombinant *E. coli* was used for the fermentation of glycerol, the amount of PHBs accumulated in the fermentation reactor was 1.47 times higher than that produced when glucose was the carbon source (Nikel et al. 2008). The difference in PHB concentrations was attributed to the difference in redox characteristics of glucose and glycerol carbon sources (Nikel et al. 2008).

DHA

Dihydroxyacetone is a three-carbon sugar and a precursor for a number of fine chemicals and pharmaceuticals such as methotrexate. DHA is used in the cosmetics industry for the production of suntans. Since DHA causes pigmentation of the skin, it is also used in the treatment of vitiligo, an autoimmune disease in which the melanocytes are destroyed and irregular white patches are formed on the skin (Fesq et al. 2001). *Gluconobacter oxydans* is used industrially for the production of DHA. Glycerol is converted to DHA by the membrane-bound enzyme GldA. At high concentrations, DHA is toxic to microbial cells, and cell viability decreases exponentially with time. Overexpressing GldA gene (*sldAB*) in *G. oxydans*, however, increased the concentration of DHA from 18–25 g/L to 30 g/L and the inhibitory effect of DHA on cell viability was also reduced (Gatgens et al. 2007).

Propionic Acid

Propionic acid is an important antifungal agent used in industrial production of cellulose-based plastics, solvents, herbicides, perfumes, flavors, thermoplastics, and so on. Propionic acid is formed from succinate via the dicarboxylic acid pathway. Propionic acid is natively produced by *Propionibacterium acidipropionici*, *Propionibacterium acnes*, and *Clostridium propionicum*. These bacteria are capable of fermenting crude glycerol to propionic acid. *P. acidipropionici* can produce up to 42 g/L of propionic acid, with a yield of 0.844 mol/mol glycerol and a productivity of 0.36 g/L/h (Barbirato et al. 1997).

Citric Acid

Citric acid is used as a flavoring and preservative agent in foods and beverages, as a water softener in detergents, and as a wax and color remover in shampoos. It is generally produced by *Aspergillus niger* and *Yarrowia lipolytica*. *Y. lipolytica* produced 11 g/L of citric acid in a glucose medium (Papanikolaou et al. 2002). When grown in a glycerol medium, this microorganism produced up to 35 g/L citrate with a yield of 0.42–0.44 g/g glycerol (Papanikolaou et al. 2002).

Prodigiosin

Prodigiosin is a red pigment produced by many strains of *Serratia marcescens* and other microorganisms such as *Pseudomonas magnesorubra* and *Vibrio psychoerythrus*. The prodigiosin group of natural products belongs to the Psychroerythrus family of tripyrrole red pigments that contain a common 4-methoxy, 2-2 bipyrrole ring system. The biosynthesis of the pigment is by a bifurcated process in which mono- and bipyrrole precursors are synthesized separately and then assembled to form prodigiosin (Boger and Patel 1988). This pigment is capable of inducing apoptosis in several cancer cell lines, except nonmalignant cells. It can be used, therefore, as a potential antineoplastic candidate. *Serratia marcescens* has been reported to use crude glycerol as a carbon source to produce up to 583 mg/L of prodigiosin (Tao et al. 2005).

Astaxanthin

Astaxanthin is a red or orange pigment usually included in marine feed to improve fish color and visual appeal. In their natural habitat, the food chain provides chemicals and nutrients

that give some species of marine fish their characteristic pink color. To retain this pink color under artificial environment, astaxanthin is often incorporated into fish feed. Astaxanthin is naturally produced by the yeast *Phaffia rhodozyma* from β -carotene via a two-step reaction catalyzed by β -carotene ketolase and β -carotene 3,3'-hydroxylase. This organism has been reported to ferment crude or industrial glycerol to produce 34 mg/L of astaxanthin (Kusdiyantini et al. 1998).

Biosurfactants, Fatty Acids, and Lipids

Biosurfactants are microbial-derived surface-active agents that can be used as emulsifiers, de-emulsifiers, wetting agents, foaming agents, functional food ingredients, and detergents in various industrial sectors such as petrochemicals, food and beverages, cosmetics and pharmaceuticals, agrochemicals, and fertilizers. They are preferred to chemical surfactants due to their biodegradability, surface properties, and low toxicity. Rhamnolipid is a type of biosurfactants synthesized by *Pseudomonas aeruginosa*. This microorganism produced 15.4 g/L of rhamnolipid during growth in a basal medium supplemented with crude glycerol (Zhang et al. 2005).

Another type of biosurfactant, called sophorolipid (SL), is produced by yeast *Candida bombicola* as an extracellular glycolipid. This glycolipid is composed of a disaccharide (i.e., sophorose) attached to a hydroxy fatty acyl moiety at the omega minus one or omega carbon atom (Asmer et al. 1988). The fatty acid (saturated or unsaturated) component of the glycolipid is composed of 16–18 carbons. The carboxyl group is either lactonized to the disaccharide ring or free as in the open chain form. Since the hydroxyl groups attached to the disaccharide ring are capable of being acetylated, the whole molecule is amphiphilic with surfactant properties. *C. bombicola* produced 9 g/L of SL in a pure glycerol medium, but when grown in glycerol waste stream obtained from a biodiesel plant, up to 60 g/L SLs was produced. This dramatic increase in SL production was attributed to low osmotic stress and presence of fatty acids in the crude glycerol (Ashby et al. 2005).

The conversion of glycerol to single cell oil (SCO) by microbes is another commercially important process due to the use of SCO as nutraceuticals, pharmaceuticals, and feed ingredients for aquaculture. Microbial lipids have similar properties to vegetable fats and oils in terms of structure and composition and hence have potential to replace them. *Y. lipolytica*, when grown in a medium containing crude glycerol, produced 8.1 g/L of SCO at the dilution rate of 0.03/h with a maximum productivity of 1.2 g/L/h (Papanikolaou et al. 2002).

Docosahexaenoic acid is an omega-3 polyunsaturated fatty acid ($\omega - 3$ PUFA) that has a protective role against cancers, Alzheimer's disease, cardiovascular diseases, schizophrenia, and so on. This compound is currently obtained from fish oil but can also be extracted from $\omega - 3$ PUFA-enriched marine algae. The extracted PUFA can be used as a food additive, pharmaceutical, and aquaculture feed ingredient. The marine alga *Schizochytrium limacinum* produced large amounts of docosahexaenoic acid when grown on either a glucose or a glycerol medium. When crude glycerol was used as a carbon source, *S. limacinum* produced approximately 4.91 g/L docosahexaenoic acid (Chi et al. 2007).

Glycerol can be chemically converted to a variety of commercially important chemicals such as propylene glycol, propanediols, and acrolein. Traditionally, glycerol is esterified to fatty acids to produce polyglycerol esters that are used either as emulsifiers in the food industry or as detergents. The price of glycerol has fallen recently, and the production of other high-value chemicals from glycerol has been explored. These include the oxidation of glycerol to glyceric acid and tartronic acid, and the production of glycerol carbonate.

Ethanol Production from Glycerol

Although a number of high-value products have been produced using glycerol, production of ethanol from glycerol provides a unique opportunity for the biodiesel industry to generate another biofuel from biodiesel waste. *E. aerogenes* HU101 isolated from methanogenic sludge produced ethanol and H₂ when glycerol was used as a substrate (Ito et al. 2005). Low amounts of lactate, acetate, formate, and 1,3-PDO were also detected as coproducts. A maximum ethanol yield of 0.85 mol/mol-glycerol and concentration of 90 mM was reported following anaerobic fermentation of glycerol (Ito et al. 2005). Although promising, *E. aerogenes* needs yeast extract and tryptone for enhanced glycerol utilization. The highest yield and productivity were achieved in a packed-bed reactor that used porous carrier matrix as support material to immobilize microbial cells (Ito et al. 2005).

Escherichia coli has been reported to metabolize glycerol fermentatively under favorable culture conditions (Dharmadi et al. 2006). Cells growing fermentatively on glycerol exhibited exponential growth at a maximum specific growth rate of 0.040 per hour (Murarka et al. 2008). Cell growth was not affected despite blocking of several respiratory processes, demonstrating the fermentation of glycerol by *E. coli*.

Pathways responsible for fermentative metabolism of glycerol in *E. coli* have been revealed (Gonzalez et al. 2008). *E. coli* mutants with disrupted respiratory genes *glpD* and *glpA* have been shown to ferment glycerol, indicating the existence of alternative pathways for the metabolism of glycerol in the absence of electron acceptors such as oxygen and fumarate. *E. coli* encodes a type II glycerol dehydrogenase enzyme, GldA, which was earlier thought to be cryptic in nature without any significant physiological role in wild-type strains (Jin et al. 1983). Nonetheless, this enzyme has the potential to oxidize glycerol into dihydroxyacetone (DHA) that can further be converted to DHAP by DHA kinase. When *gldA* (encoding GldA) and *dhaKLM* (encoding DHA kinase) mutants were evaluated for their ability to ferment glycerol, they failed to do so. This observation underscores the active role of GldA and DHA kinase in the glycerol fermentation pathway. In addition to this oxidative pathway for metabolizing glycerol, a parallel reductive pathway acting as an electron sink was also discovered in the form of 1,2-PDO (Figure 6.6). Disruption of the 1,2-PDO synthesis pathway decreased cell growth on glycerol, while overexpression of this pathway led to cell growth without any supplementation of rich media (Gonzalez et al. 2008; Murarka et al. 2008).

Enhancement of Yield and Productivity of Ethanol

Identification of pathways and environmental conditions affecting the metabolism of glycerol under anaerobic conditions by wild-type *E. coli* provide opportunities to manipulate the microorganism for enhancement of ethanol yield and productivity. Fermentation of glycerol to either ethanol and H₂ or ethanol and formate is one of the most effective ways of exploiting the reduced property of glycerol for bioproducts production.

Ethanol is predominantly produced following glycerol fermentation by *E. coli*. The product mixture, however, contains succinate, acetate, and formate. Succinate and acetate are competing byproducts because some substrates that would have been used for the production of ethanol are diverted toward the production of succinate and acetate (Murarka et al. 2008), resulting in decreased ethanol yield. Blocking pathways for succinate and acetate synthesis are possible ways to enhance ethanol yield (Figure 6.7). Formate is a non-competing

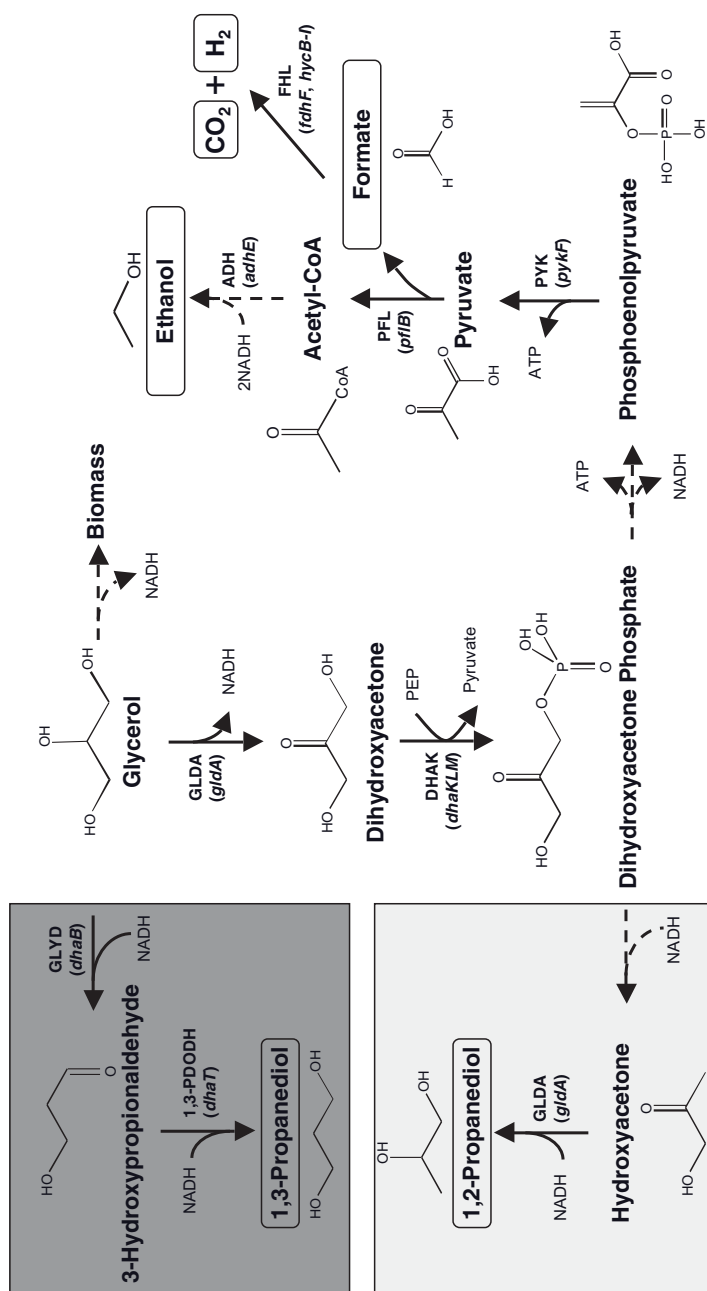


Figure 6.6. Ethanol-H₂ production pathways in *Escherichia coli* and *Enterobacter anaerogenes*. Light-shaded box (1,2-propanediol pathway) and dark-shaded box (1,3-propanediol pathway) represent ancillary pathways in *E. coli* and *E. anaerobium*, respectively. Enzymes catalyzing relevant steps and their corresponding genes (in bracket) are shown. Broken arrows signify more than one step in the pathway. GLD, glycerol dehydrogenase; DHAK, dihydroxyacetone kinase; PYK, pyruvate kinase; PFL, pyruvate formate lyase; FHL, formate hydrogen lyase; ADH, alcohol dehydrogenase; GLYD, glycerol dehydratase; 1,3-PDODH, 1,3-propanediol dehydrogenase.

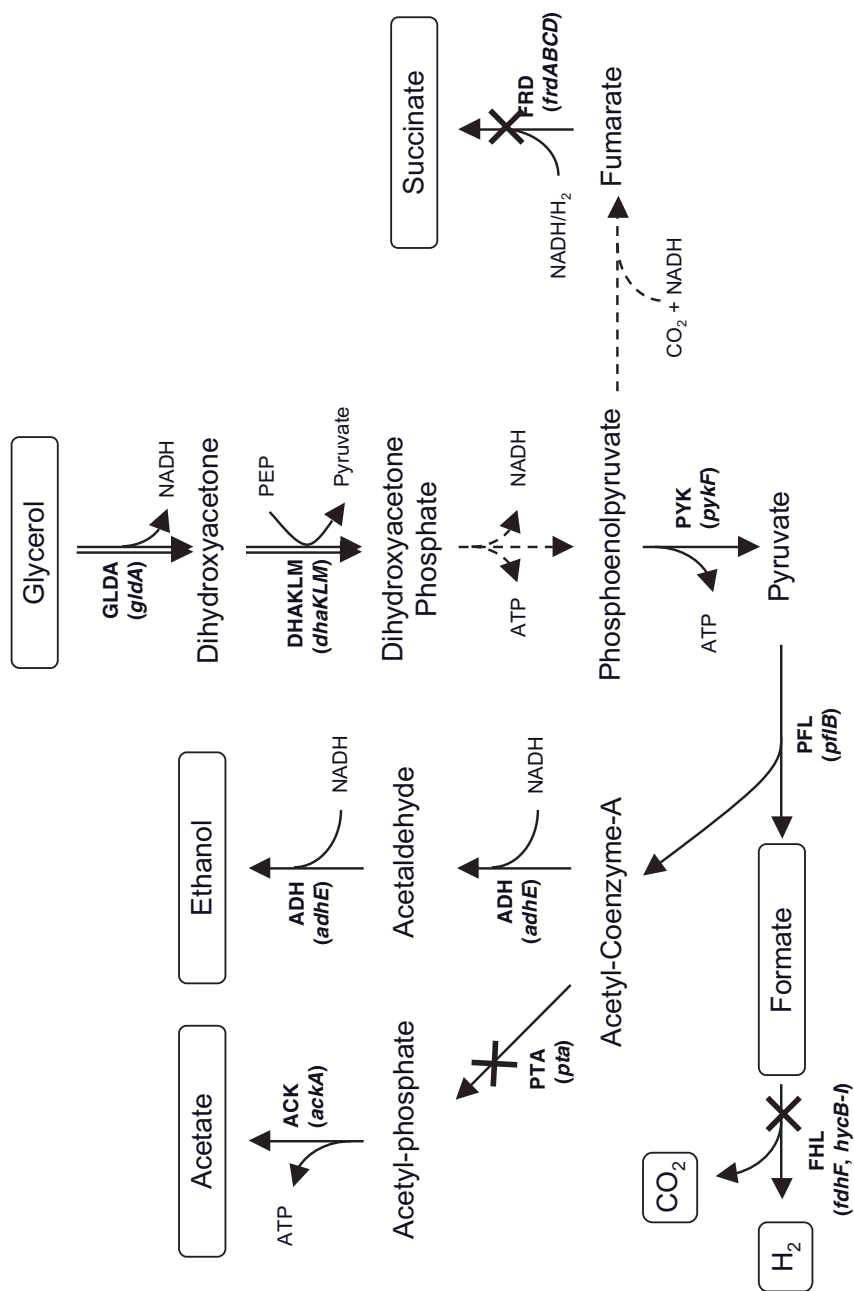


Figure 6.7. Metabolic engineering of *Escherichia coli* fermentative pathway. Fermentation of glycerol by *E. coli* leads to the production of ethanol, acetate, succinate, and formate. Pathway engineering was carried out to enhance the yield and ethanol productivity. Double arrows and cross represent overexpression and deletion of genes, respectively. Broken lines represent multiple steps. Substrate and fermentation products are indicated in the box. Relevant enzymes and their corresponding genes are also shown. **ADH**, acetaldehyde/alcohol dehydrogenase; **ACK**, acetate kinase; **DHAK**, dihydroxyacetone kinase; **FHL**, formate hydrogen lyase; **FRD**, fumarate reductase; **GldA**, glycerol dehydrogenase; **PFL**, pyruvate formate lyase; **PTA**, phosphate formate lyase; **PYK**, pyruvate kinase.

coproduct of fermentation of glycerol to ethanol. Strain engineering and optimal culture condition techniques provided us with the expertise needed to embark on two independent strategies to enhance ethanol yield and productivity during fermentation of glycerol to ethanol by *E. coli*. The two strategies are: (1) coproduction of ethanol and H₂, and (2) co-production of ethanol and formate.

For the ethanol–H₂ production pathway, we carried out two mutations in *E. coli* to disrupt genes that encode fumarate reductase (FRD) and phosphotransacetylase (PTA; Figure 6.7). FRD and PTA are two key enzymes involved in the production of succinate and acetate, respectively. The resultant strain, SY03, produced an almost equimolar amount of ethanol and hydrogen and yield comparable to theoretical maximum of 1 mol of each product per mole of glycerol (Yazdani and Gonzalez 2008). To facilitate coproduction of formate and ethanol, another mutation was introduced in the gene (*fdhF*) encoding component of FHL in the strain SY03 (Figure 6.7). FHL is responsible for the oxidation of formate into H₂ and CO₂. This triple mutant strain, called SY04, produced exclusively ethanol and formate at yields of 92%–96 % of the theoretical maximum.

The strategies used in the generation of these mutants led to a decrease in the growth rates of *E. coli* mutants (Yazdani and Gonzalez 2008). The overexpression of GldA and dihydroxyacetone kinase (DHAK), which are responsible for converting glycerol into glycolytic intermediate DHAP, was assessed for improving the growth rate of *E. coli* mutants. We overexpressed GldA and DHAK separately and in combination with plasmids pZSgldA, expressing GldA, pZSKLM expressing DHAK, and pZSKLMgldA expressing both GldA and DHAK to improve the rate of glycerol fermentation (Yazdani and Gonzalez 2008). Transformation of these plasmids in *E. coli* MG1655 led to a 10–20-fold increase in GldA and a 5–6-fold increase in DHAK activities. When the effect of overexpression of GldA and DHAK genes in *E. coli* MG1655 was tested on glycerol fermentation, individual overexpression of either GldA or DHAK did not have a significant effect on glycerol fermentation. Simultaneous overexpression of GldA and DHAK from plasmid pZSKLMgldA led to a 3.4-fold increase in the amount of glycerol fermented in *E. coli* MG1655. Overexpression of GldA and DHAK in the host SY04 with mutations in three genes, *frdA*, *pta*, and *fdhF*, for the coproduction of ethanol–formate led to the production of ethanol and formate at maximum volumetric rates of 3.58 and 3.18 mmol/L/h, respectively.

Further experiments involving various mutants confirmed role of both respiratory and fermentative pathways of glycerol utilization under microaerobic condition (Durnin et al. 2009). Enzymes involved in the respiratory pathway of glycerol metabolism, glycerol kinase (GlpK), and glycerol-3-phosphate dehydrogenase (GlpD) exhibited higher activities in the initial aerobic phase of glycerol utilization. The transition to microaerobic conditions, characterized by undetectable amounts of dissolved oxygen, resulted in a 2.5-fold increase in glycerol-3-phosphate dehydrogenase activity and a 10-fold increase in both GldA and DHAK activities. The GlpK–GlpD pathway predominated during the early phase of fermentation, while the GldA–DHAK pathway predominated during the later stage of cultivation. Under microaerobic conditions, the engineered strains were able to utilize glycerol to produce ethanol and hydrogen, or ethanol and formate in basal media.

Conclusions and Future Outlook

Considering the worldwide surplus of crude glycerol and the need to find new uses for this cheap abundant carbon source, the use of anaerobic fermentation to convert low-value

crude glycerol streams generated during the production of biodiesel to value-added products represents a promising step toward achieving an economically viable biodiesel industry. A number of organisms are able to ferment glycerol to different bioproducts with a wide range of applications. The success of these strategies will depend on the use of robust microorganisms that are amenable to industrial applications.

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Chapter 7

Farm-Gate to Plant-Gate Delivery of Lignocellulosic Feedstocks from Plant Biomass for Biofuel Production

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Abstract

This chapter presents the logistics of delivering cellulosic biomass feedstock from the farm gate to the plant gate. Unlike the more familiar starchy feedstock and oilseed such as corn and soybean, which can be cost-effectively delivered to the plant gate for processing at the commercial scale, cellulosic biomass presents unique handling challenges at the commercial scale, which make them expensive to utilize. In the Introduction section of this chapter we discuss the essential components of the logistics chain and the unique logistical needs of lignocellulosic biomass as compared with starchy grain feedstocks. Then we discuss on-farm logistics of biomass harvest and delivery, which include biomass availability and distribution, harvest and collection, preprocessing operations, transport, economic, energy inputs, and carbon emissions. After that we discuss the logistics of handling feedstock at the plant gate, we examine the operations used in grain elevators, and we discuss the envisaged parallel operations that would occur in a biorefinery processing lignocellulosic biomass. Next we discuss related agricultural logistics operations (cotton harvest logistics, sugarcane harvest logistics, and fuel chip harvest logistics) and their application to biomass. A comparison of herbaceous fiber and grain logistics chain is also presented. The last section briefly presents a systems approach to feedstock logistics, discussing some unique opportunities to save cost and energy by integrating unit operations throughout the logistics chain.

Introduction

Lignocellulosic feedstocks from plant biomass such as grain plant residues (corn stover, wheat straw, rice straw, etc.), energy crops (switchgrass, canary reed grass, miscanthus, etc.), and wood (residues, wood chips, hybrid poplars and willows, etc.) are currently being investigated for use in the production of second-generation advanced fuels (cellulose ethanol, FT-diesel, etc.) and for biopower either fired alone or co-fired with coal. As compared to

corn-grain-for-ethanol, their utilization for energy production does not compete with the demand for agricultural feedstocks such as grain for food and feed production. The increased production of corn ethanol in the United States has raised a number of concerns in recent times, the major one being the moral and social consciousness of using a food crop for fuel that could potentially drive up world food prices and create a hunger crisis in poor regions of the world. Another obvious reason for the use of lignocellulosics is because it would be practically impossible to produce the mandated fuel ethanol volumes with grain crops alone, even if the entire grain crop produced in the United States were converted to ethanol. Additionally, the Energy Independence and Security Act of 2007 (WhiteHouse News Releases 2007) sets a mandatory Renewable Fuels Standard (RFS) for the production of 36 billion gallons of fuel ethanol by 2022, of which 15 billion must be produced from cellulose. While there is no commercial scale cellulose ethanol plant, there are commercial power plants in North America, Europe, and Asia that have some experience using biomass on a large scale.

Commercial scale utilization of lignocellulosic biomass is not a trivial task and is quite different from the use of grain. The logistics and handling cost of feedstock can be very expensive and is one of the major reasons for the high cost of producing liquid fuels and power from lignocellulosic feedstocks. In corn stover to ethanol production, feedstock and handling cost together can make up as much as 36% of the production cost (Ruth et al. 2002). The three diverse types of biomass mentioned previously, while chemically the same, are quite different in their times of harvest/collection, method, and physical characteristics. This means that the unique differences of these feedstocks need to be considered when designing an effective biomass logistics system. Once the feedstock is ready for harvest and collection, field machinery must be scheduled for harvesting the feedstock within a narrow window of opportunity usually from a few weeks to 3 months. Harvest is followed by transportation to on-farm storage, preprocessing, or biorefinery plants. Sustainable supply of feedstock from on farm storage must be delivered to the biorefinery year round to meet about 350 days of production schedule while maintaining an average of at least 10 days inventory at the biorefinery. The logistics of all these operations must be coordinated with the goal of delivering the least cost feedstock of the specified quality at the plant gate.

The design and operation of efficient feedstock delivery systems are vital to reducing the cost of feedstock/handling for commercial bioenergy production from lignocellulosics. Feedstock handling involves field harvest and collection, storage, preprocessing, transportation and handling/delivery at the biorefinery. In the next two sections, we will discuss these operations in two segments: (1) field harvest/collection, preprocessing, and transport to the biorefinery and (2) handling/delivery of inbound feedstocks at the biorefinery. The third section will present the principles required for an efficient logistic system for biomass, illustrated by several commercial operations making use of existing systems in U.S. agriculture. Finally, a systems integration approach will be presented as a way of approaching feedstock delivery by integrating it within the production system of the bioenergy conversion process.

On-Farm Logistics of Biomass Harvest and Delivery

Introduction

Typically, the feedstock cost constitutes about 35%–50% of the total production cost of ethanol or power. The actual percentage depends upon biomass species, yield, location, climate, local economy, and the type of systems used for harvesting, gathering and packaging,

processing, storing, and transporting of biomass as a feedstock. The following is a list of feedstock requirements to ensure the success of biorefineries.

- Identify quantities, quality of biomass, delivery costs for the year-round supply of biomass.
- Conduct resource assessment considering mix of available biomass species, annual yield variations, environmental factors, seasonality, and competitive demands for biomass.
- Optimize for the least cost equipment and infrastructure for timely harvest, densifying, storing, and transporting of the biomass.
- Develop regional and national strategies for locating biorefineries and organizing supply chains with respect to biomass cost and availability.

Figure 7.1 shows a diagram of biomass-to-product thread from production to biorefinery. The type of biorefinery may range from biomass to heat and power or to production of chemicals and liquid fuels. Biomass production can be from agricultural and forestry activities, and municipal and industrial wastes. The activities within the large oval identify the current and probable future biomass supply enterprises. Biomass is collected in a distributed system at the farm or at the forest level. The collected biomass is transported either a short distance (0–100 km) or a long distance, hundreds of kilometers for storage and/or preprocessing. Preprocessing may include one or a combination of several of size reduction, fractionation, sorting, and densification. The storage of wet biomass may also impart biochemical and physical modifications to the biomass. We call this as in-store preprocessing. The pre-processed biomass is transported to a biorefinery where it is fed directly into the conversion reactor.

The arrows on the diagram show the flow of material and information. The information flow (lines with arrows between unit operations) from the biorefinery to a biomass supply enterprise includes quality specifications for biomass, that is, moisture content, particle size,

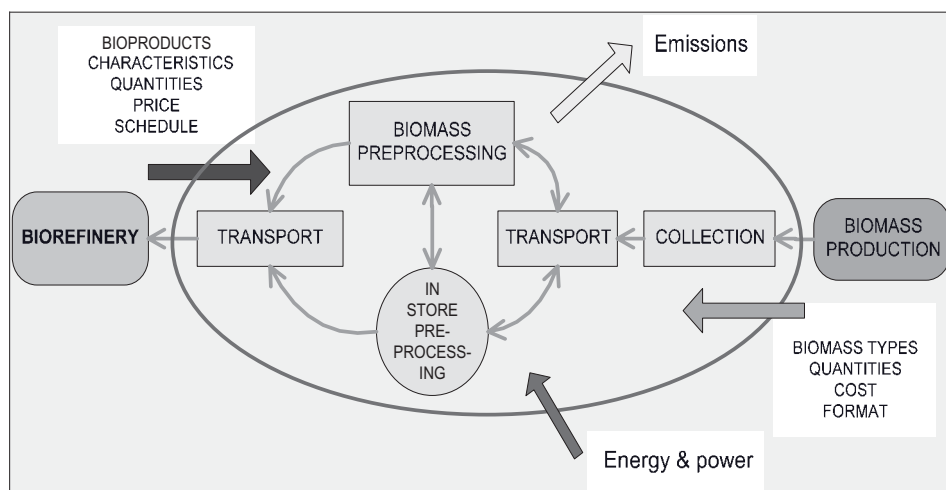


Figure 7.1. Biomass supply enterprise as an integral part of biomass to biorefinery chain.

composition (cellulose, hemicellulose, lignin, ash content, chlorines, silica, etc). Important information for logistics includes quantities and delivery schedules and price. In response to demands, the production side provides biomass to the supply system (biomass types, quantities, format, and cost). The supply system uses energy and power to collect, preprocess, and transport biomass. The system will give off emissions that need to be minimized.

The objective of this section is to present an analysis of the cost of collecting and handling of biomass throughout the entire supply chain using several collection, storage, and transport options. The Integrated Biomass Analysis and Logistics (IBSAL) model is used for this analysis. The section also discusses energy input to the model and carbon emissions from powered equipment used in the supply chain.

Biomass Availability and Distributions

Biomass Yield

For agricultural residues, especially straws and stover, the quantity of biomass is estimated from grain yield and the ratio of straw or stover to grain. In converting yield from grain to biomass, we need to consider definition of bulk density (test weight) of grain and moisture content at which the bulk density is given. Once the gross yield of biomass is calculated (usually and preferably expressed in dry mass t) then we discount this value depending on the following factors:

- Minimum recommended mass to be left on the field for soil conservation. This amount can be calculated from detailed soil loss models or from recommended soil coverage.
- Field losses of equipment working on the biomass during harvest and postharvest operations. These losses result in reduction of mass because of breakage and leaf loss.
- Losses because of elements in the field and in storage. These losses can be physical and biochemical reactions in the biomass that is left in the field or in storage.

In the case of a dedicated energy crops such as switchgrass, total biomass yield depends upon the time of harvest. In summer the biomass is green and high in moisture; later in the fall the biomass is in a mature stage or senescence and usually low in minerals (ash content); and in the following spring the biomass is often dry, brittle, and has lost some of its mass because of snow and wind. For instance, if the land is furrowed the height of cut must be kept high (more than 150 mm) in order to minimize contamination resulting from dirt and surface soil.

Values listed in Table 7.1 are not precise and are given here for demonstration purposes. The estimated net yield of straw is 1.8 t/ha, stover 3.7 t/ha, and switchgrass 6.75 t/ha.

Supply Area

The supply area is calculated from annual demand for biomass and the net yield of biomass. Table 7.2 shows the area of cultivated land to provide 500,000 t of biomass annually. To calculate the total gross area, we then have to apply at least three factors: (1) fraction of the land under biomass cultivation; (2) how often (harvested every year, every other year, etc.) the producer will supply the biomass; and (3) the sector ratio assuming a circle for the supply area. Table 7.3 shows that the total area and the radius of supply circle increases substantially depending upon the supply factors (1)–(3). In this analysis, the competition of biomass for animal bedding and feeding and other industrial usage (press board, biofuel production, etc.) has not been considered.

Table 7.1. Calculations of the net yield for three biomass types.

Biomass Type	Yield ^a Grain (bu/ac)	Dry Grain (t/ha)	Straw/Grain Ratio ^b	Gross Yield (t/ha)	Max Fraction Removed for Soil Fertility k_1	Fraction Machine can Remove k_2	Estimate of Losses from Harvest to Biorefinery k_3	Net Yield (t/ha)
Wheat straw	60	3.5	1.3	4.6	0.5	0.75	0.20	1.82
Corn stover	150	8.1	1.0	8.1	0.7	0.75	0.35	3.68
Switchgrass	—	—	—	10.0	0.8	0.75	0.10	6.75

^aTest weight for wheat at 60lb/bu at 14% m.c. Test weight for corn at 56lb/bu at 15.5% m.c. Weights are in dry mass.

^bThe straw/grain ratio is the yield of grain to that of its biomass (stalks and leaves); that is, dry weight of grain/dry weight of plant biomass.

Table 7.2. Calculation of cultivated area and the supply area for biomass (all weights are in dry mass).

Biomass Type	Net Yield (t/ha)	Annual Demand	Cultivated Area (ha)	Sectors in which Crop Is Grown (n)	Fraction under Crop	How Often (Years) Biomass Is Available	Total Area (ha)	Supply Radius (km)
Wheat straw	1.822	500,000	274,403	1.33	0.2	3	5,488,062	132
Corn stover	3.677	500,000	135,983	1.67	0.3	2	1,510,919	69
Switchgrass	6.750	500,000	74,074	2.00	0.1	1	1,481,481	69

Supply Schedule

Harvest of crop residue follows grain harvest. The grain moisture content at its physiological maturity may be in the range of 30%–40%. As soon as grain reaches this moisture, harvest will start, but not at once. Initially, a few harvests will start but the pace of harvest will pick up as the season progresses. Once the peak harvest passes, the pace of harvest slows down. In northern climates and for corn that often grows in summer, the harvest is completed before the cold temperatures set in and the work in the field becomes impossible due to rain or snow. The harvest season for grain crops ranges from 4 to 10 weeks.

In the case of switchgrass, the crop can be harvested twice a year with roughly 70% of the yield obtained from the first cut and 30% of the yield from the second cut. The yield and mass ratio of the first and the second cut drop for mid-western and northern regions of the United States (Vogel et al. 2002). In biomass supply analysis, harvesting twice a year would be expensive due to machinery use and low biomass yield. Therefore, it is appropriate to use a single harvesting approach for switchgrass, which usually commences from August 1 and continues for the next 3 months (August, September, and October). The harvest activity in the Midwest stops when daily average temperature is below -5°C . In the Upper Southeast, harvest can proceed through the winter until the end of March on days when soil conditions are such that equipment can operate.

Biomass Harvest and Collection

Harvest and collection constitutes gathering and removing the biomass from field. The operations depend upon the state of biomass on the field. This includes the type of biomass (grass, woody, or crop residue). The moisture content and the end use of biomass also affect the way biomass is collected. For crop residue, the operations need to be organized in companion with the grain harvest. In this section, we examined advanced systems that may be used to gather biomass residues.

Harvesting

For crop residues, grain harvest most probably will take the center stage. All other operations, such as residue management and collection, take place after grain is harvested. This situation may change in future but at the present time this is the case. Figures 7.2 and 7.3 show the present and future scenarios for harvesting crop residues. Future scenarios are highlighted in the larger boxes encompassing more than one unit operation. In the case of corn, a combine takes a small portion of the corn stalks. The majority of the corn stalk left in the field is anchored to the ground. The stalks need to be shredded before a baler can pick them up. Figure 7.3 also shows the use of new stripper headers for harvesting grain. Stripper headers strip the grain from the stalk and leave the stalks standing in the field. The straw stalks are then cut and placed in a swath for baling.

Loafing is an attractive option, because collection, densification, and transport to the side of the farm can be done with a single equipment unit. Loafing of stover is practiced in Iowa but its performance with straw and dedicated crops is unknown. Corn stalk moisture is high especially early in the season. One option is to chop the high moisture stover and store it in a bunker silo as silage. This option is under investigation.

Cutting and Field Drying

In the case of straw the biomass is generally dry. For stover the leftover biomass after grain harvest may be dry or wet. In the case of switchgrass, depending on the time of harvest, the

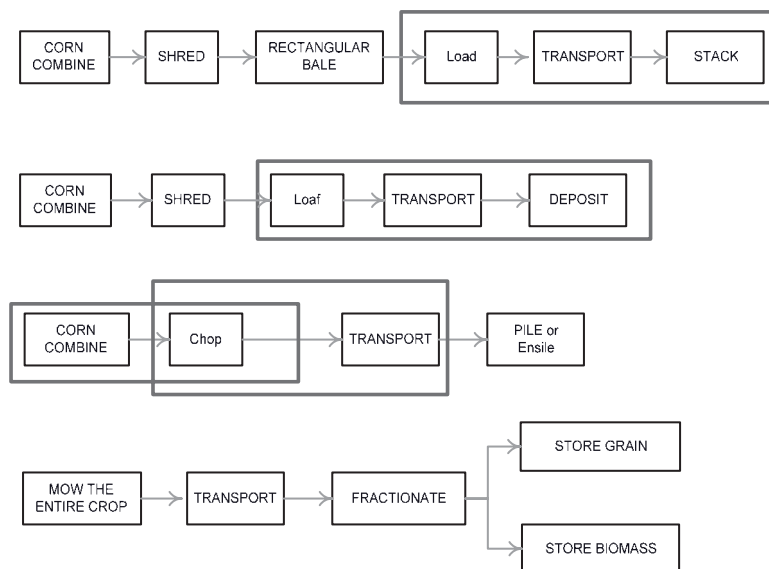


Figure 7.2. Options for collecting and stacking stover.

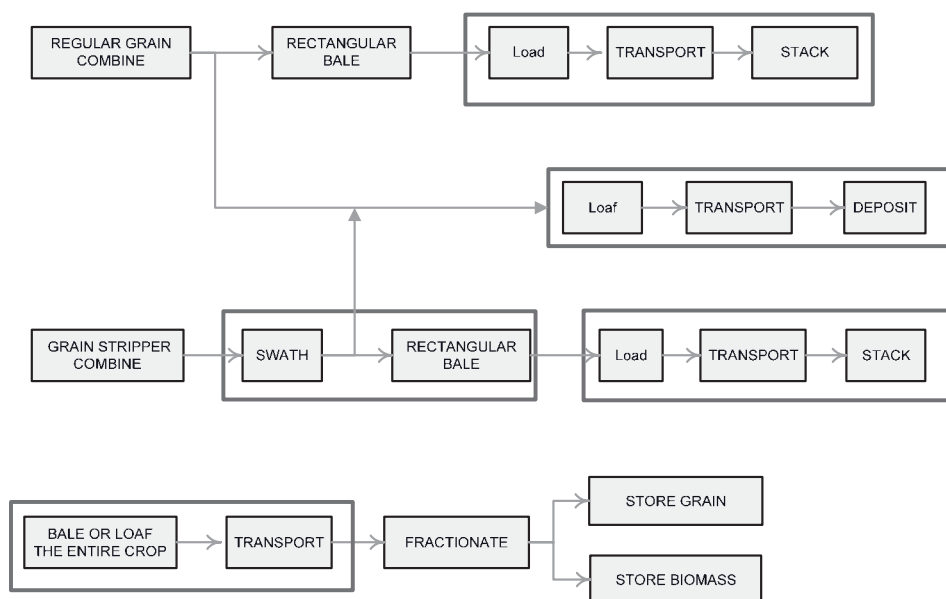


Figure 7.3. Options for collecting and stacking wheat straw.

biomass may be wet as much as 80% moisture content in the middle of summer to a low of 20% at the end of the growing season. A field shredder is used to cut the material to pieces and spread on the field for drying. For green switchgrass, mowing may be combined with conditioning, where the cut material passes through two or more rollers. The rollers break the green stems at several points along the stalk. The bruise and cut provide escape routes for the plant moisture to evaporate quickly. Various degrees of maceration or severe bruising and cutting (super conditioning) have been developed in recent years.

When switchgrass is dry and standing in the field, a mower would be adequate to cut the plant and place it in a swath for immediate baling. No conditioning or maceration is needed. This statement was validated by Venturi et al. (2004) who recommend mowing and conditioning during early season but only mowing late in the season as the moisture content of the plant decreases. But, they also found that round baling late in the season is difficult due to the toughness and lack of pliability of the straw.

For wheat, cutting is not required as the height of cut can be adjusted during combining. In stripper header combining, standing stalks are cut and windrowed for baling. For most cases, straw is of low moisture content at the time of grain harvest or immediately after grain harvest. Operations to expedite field drying of straw may not be needed.

For corn stover, grain and stalk are at different moisture content during harvest. Figure 7.4 is a plot of stover moisture content and grain moisture content after the kernel has matured to 40% moisture content (Sokhansanj et al. 2008). Stover moisture content initially at more than 75% (w.b.) drops to 10% toward the end of harvest season. Special operations are needed to deal with the variation in moisture content. Shredding the stover and spreading it with the combine accelerates field drying. The spread material then has to be raked into windrows for efficient baling. Many operations use a flail shredder to shred the broken stalks while gathering the shredded material in a windrow in a single operation.

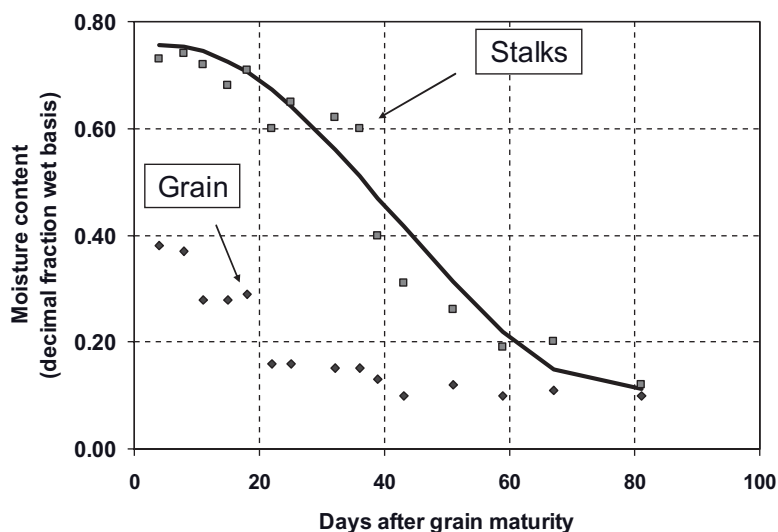


Figure 7.4. Moisture content of corn stalks (solid line and circles) and of the grain (diamonds) after grain maturity date (Sokhansanj et al. 2006).

Collection

Collection is defined as operations for collecting, packaging, and transporting biomass to a nearby site for temporary storage. The most conventional method for collecting biomass is baling. Bales are in the form of either rounds or squares. Round bales are popular on most U.S. farms (Cundiff 1995, 1996; Bransby and Downing 1996; Cundiff and Marsh 1996; Cundiff and Shapouri 1997; Cundiff et al. 2004). Limited experience with using round bales for biomass applications indicates that round bales are not suitable for large scale biomass handling. Because of their round shape, round bales tend to deform under static loads in a stack. Bales that are not perfectly round cannot be loaded onto trucks to form a transportable load over open roads. Experience with switchgrass harvest at the Chariton Valley Co-Firing project in Iowa (CV—RC&D. Chariton Valley Biomass Project Design Package 2002; Miles 2006) showed that variations in the density of round bales were the cause of uneven cuts and erratic machine operation during the de-baling process.

Baling

Large square bales are made with tractor pulled balers. Large square bales are currently made either in dimensions $1.2\text{ m} \times 1.2\text{ m} \times 2.4\text{ m}$ ($4' \times 4' \times 8'$) or $0.9\text{ m} \times 1.2\text{ m} \times 2.4\text{ m}$ ($3' \times 4' \times 8'$). A bale accumulator is pulled behind the baler that collects the bales in groups of four and leaves them on the field. At a later date when available, an automatic bale collector travels through the field and collects the bales. The automatic bale collector travels to the side of the road and unloads the bales into a stack. If the automatic bale collector is not available bales may be collected using a flat bed truck equipped with a front-end bale loader. A loader is needed at the storage site to unload the truck and stack the bales. The stack is tarped using a forklift and manual labor.

Loading

Mowing, conditioning, and raking operations are identical to those for baling. When biomass is dry, a loafer picks the biomass from windrow and makes large stacks of about 2.4-m wide, up to 6-m long and 3.6-m high (SAF 1979; FMO 1987). The roof of the stacker acts as a press pushing the material down to increase the density of the biomass. Once filled, loafer transports the biomass to storage area and unloads the stack. The top of the stack gets the dome shape of the stacker roof and thus easily sheds water. The loafer has been used for hay and for corn stover. It was used for experimental wheat straw in Idaho. To the knowledge of the authors, the loafer has not been used for switchgrass, so its practical performance is not known at this time.

Dry Chop

In this system a forage harvester picks up the dry biomass from windrow and chops it into smaller pieces (2.5–5.0 cm). The chopped biomass is blown into a forage wagon traveling along side of the forage harvester. Once filled, the forage wagon is pulled to the side of the farm and unloaded. A piler (inclined belt conveyor) is used to pile up the material in the form of a large cone.

Wet Chop

In this system, a forage harvester picks up the dry or wet biomass from the windrow. The chopped biomass is blown into a forage wagon that travels along side of the harvester. Once filled, the wagon is pulled to a silage pit where biomass is compacted to produce silage (Luginbuhl et al. 2002). For silaging dry corn stover, water is added to create silaging moisture content. To the knowledge of the authors, no literature is available for silaging dry switchgrass. Work is in progress for silaging corn stalks and wheat straw.

Whole Crop Harvest

The last rows in flow diagrams in Figures 7.2 and 7.3 show harvesting and collecting the entire crop that includes straw and grain in a single operation. The entire material is transferred to a central location where the crop is fractionated into grain and biomass. The whole crop harvesting and fractionation concept has been researched for many years (Buchele 1976). A whole crop wheat harvester was developed in Sweden in the early 1980s (Lucas 1982) at a cost of more than \$5 million. The self-propelled machine was able to harvest the entire crop, thresh and clean the grain, and bale the straw, all in one step. Recent efforts by Quick and Tuetken (2001) have been reported to develop a whole crop harvester and transporter for corn.

The McLeod Harvester (St. George 2000) developed in Canada fractionates the harvested crop into straw and graff (graff is a mixture of grain and chaff). The straw is left on the field. Grain separation from chaff and other impurities take place in a stationary system at the farmyard. The new machine is credited with higher capacity and efficiency than current grain combines. PAMI (1998) conducted an economic analysis to show that whole crop baling resulted in the highest net return among six different systems including McLeod harvester. For the whole crop baling, the crop (wheat) was cut and placed in a windrow for field drying. The entire crop was then baled and transported to the processing yard. The bales were unwrapped and fed through a stationary processor that performed all the functions of a normal combine. The straw was then rebaled.

Biomass Preprocessing

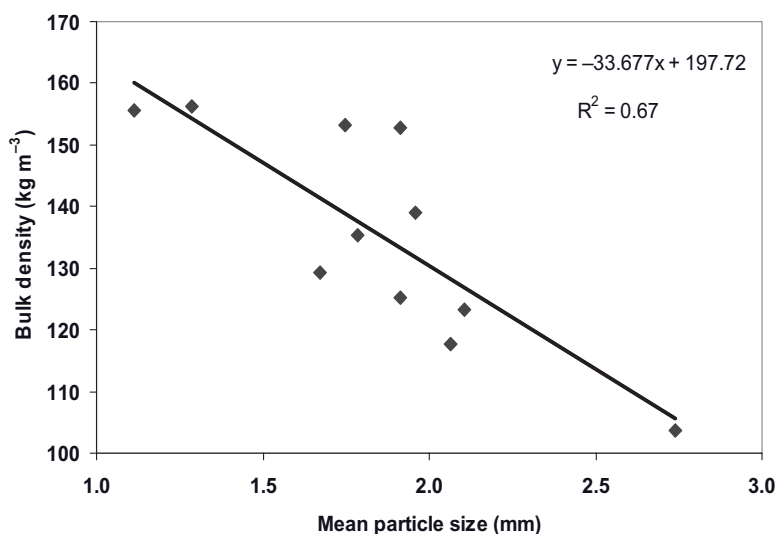
Preprocessing of biomass is an important step in preparing and supplying biomass to a bio-refinery. Literature indicates that the particles used for hydrolysis and subsequent fermentation should be in the range of 2 mm. For pulping applications size ranges should not be less than 20 mm and not more than 40 mm. For pyrolysis, particle size influences the speed of the pyrolysis. For fast pyrolysis where bio-oil are produced, the smaller the particle is the more efficient the process becomes because of high rate of heat transfer. For slow pyrolysis like gasification and charcoal making, the size of particle can be as big as 50 mm where the process of heat treatment is very slow. For pelletization of biomass to small diameter pellets, a particle size in the range of 1–2 mm is preferred. For larger cube sizes (10–30 mm) the size of particles can increase.

Loose-cut biomass has a low bulk density ranging from 50 to 120 kg/m³ depending on the particle size (Table 7.3). In case of chopped and ground biomass, the bulk density can be increased substantially (~25%) by vibrating the biomass holder (e.g., truck box, container). To increase density, the biomass must be mechanically compacted (Sokhansanj et al. 1999). When biomass is densified to briquettes, cubes, or pellets, densities in the range of 300–700 kg/m³ can be obtained.

Table 7.3. Bulk density of granulated biomass.

Form of Biomass	Shape and Size Characteristics	Density (kg/m ³)
Chopped biomass	20–40 mm long	60–80
Ground particles	1.5 mm loose fill	120
Ground particles	1.5 mm pack fill with tapping ^a	200
Briquettes	32 mm diameter × 25 mm thick	350
Cubes	33 mm × 33 mm cross-section	400
Pellets	6.24 mm diameter	500–700

^aBiomass is spread into the container while tapping the container.

**Figure 7.5.** Bulk density versus mean particle size of biomass.

Pellets are usually in the form of a hardened biomass cylinder, 4.8–19.1 mm in diameter, with a length of 12.7–25.4 mm. Pellets are made by extruding ground biomass through round or square cross-sectional dies. The unit density of pellets (density of a single pellet) is 960–1120 kg/m³. Bulk density of pellets may be as high as 750 kg/m³. Cubes have a lower density than pellets. Typical bulk density of cubes range from 450 to 550 kg/m³ depending upon the size of cubes.

Operations to Produce Dense Biomass

Densification involves compacting loose density biomass in a die into a solid compact such as a briquette or pellet. Biomass arrives at the plant in chops or bales. The bales are cut into short pieces using a hydraulic piston pressing the hay against a grid of knives. The bales can also be shredded using a roller and knife arrangement. If the moisture is more than 15%, the chopped biomass is dried in a drum dryer.

Figure 7.5 shows the relationship between particle size and bulk density of biomass for an industrial grinder. The spread in data can be attributed possibly to variations in actual particle

size distribution in various size groups. Note that in this particular example size groups vary from 1 to 3 mm. The bulk density for 2.5 mm is slightly more than 100 kg m^{-3} . The bulk density increases to more than 160 kg m^{-3} as particle size decreases to less than 1 mm. Table 7.3 indicates that bulk density can be increased by almost 25% by tapping (vibrating) the container.

In preparation for pelleting, the dried chops are ground in a hammer mill. For cubing, the chops are not ground. For pelleting, the ground biomass is mixed with saturated steam—in a paddle mixer located on top of the mill. Steam heats and moisturizes grind biomass. For cubing, small quantities of water are added to biomass. The steam or water acts as a lubricant to enhance binding. The moisture content of mash before pelleting is usually in the 10% range and that of chops before cubing is 12%.

Pellet mills are equipped with a large diameter short screw, a die ring, and from one to three press rolls. The feed screw pushes the biomass uniformly toward the openings in the die ring. Press wheel forces the feed through the die openings in the ring. The pressures in the mill range from 24 to 34 MPa (Tabil et al. 1997). Pellets and cubes exit the mill warm and moist. They are cooled and dried to a moisture content of roughly 10% for cubes and 8% for pellets. The cooled pellets and cubes are stored under roof in a flat storage or in a hopper bottom silo. Pellets and cubes are loaded into rail cars or trucks using a front-end loader or from self-unloading overhead bins.

In some cases, the preprocessing of biomass may consist only of grinding (Mani et al. 2006). The grind will have a bulk density of 180 kg/m^3 in the truck box. This density is suitable for short hauls. For longer hauls and long term storage, it is preferred to densify biomass to pellets or cubes.

Densified biomass requires less area and volume for storage and transport than loose biomass. In addition to savings in transportation and storage, densified biomass lends itself to easy and cost-effective handling. Dense cubes and pellets have the flowability characteristics similar to those of cereal grains. Bulk handling equipment for granular material is well developed and available commercially (Fasina and Sokhansanj 1996).

Modes of Biomass Transport

Numerous factors influence the size and mode of transportation. A few of these factors that the authors believe are most important are listed as follows:

- The maximum rate of biomass supply to biorefinery (t/hour)
- Form and bulk density of biomass (t/m^3)
- The distance biomass has to travel to reach to biorefinery (km)
- Transportation infrastructure (equipment, roadways, waterways, railways) available between the points of biomass dispatch and biorefinery

Transport Equipment

Transport equipment is primarily concerned with loading and unloading operations and transferring biomass from storage and preprocessing depots to a biorefinery. Transport modes include truck, train, barge, ship (ocean freighter) and pipeline. Moving feedstocks from one location to another might involve more than one of these modes of transport. The above factors determine which one of these modes or combinations of modes will suit a particular biorefinery. Truck transport and for a few cases train transport may be the only modes of transport. Barge, ship, and pipeline transport, and often train transport require truck transport

as well. Trucks interface with trains at the loading and unloading facilities of a depot or processing facility. Barge, ship, and pipeline require interfacing with train and/or truck transport at major facilities either on land or at the shores.

Physical form and quality of biomass has the greatest influence on the selection of equipment for the lowest delivered cost possible. In many transport instances, the rates are fixed for a distance and for a size of container independent of mass to be transported. A higher bulk density will allow more mass of material to be transported per unit distance. Truck transport is generally well developed and is usually the cheapest mode of transport but it becomes expensive as travel distance increases.

Pipeline transport is the least known technology for transport of biomass feedstocks and may prove to be the cheapest and safest mode of transport—but perhaps in a distant future. It is envisioned that biomass could be transported by pipeline in the form of a slurry mixture. Upstream equipment includes receiving, slurry making, and initial pumping. The elements along the pipeline are the booster pumps and, at the end, the equipment for draining the biomass from the carrier liquid (Kumar et al. 2005). Unlike truck and train transport, there is an economy of scale for pipeline transport. A larger diameter pipe has a lower friction and thus lower pumping cost. It is also proposed to make dense granules of biomass impervious to water or other liquids for efficient loading and long-distance transport.

Logistics

Logistics of biomass supply involves an orderly flow of biomass from farm to factory. Figure 7.6 shows at least five options for the supply chain configurations to transfer baled biomass to biorefinery. In options 1 and 2, the baled biomass is transported directly from farm or from stacks next to the farm to the biorefinery. Biomass may be minimally processed (i.e., ground) before being shipped to the plant. In this case the biomass is generally supplied from the stacks where the biomass will be minimally processed. The biomass is trucked directly from farm to biorefinery if no processing is involved.

The supply options 3 and 4 transfer the biomass to a central location where the material is cumulated and dispatched to biorefinery later on. While in the depot, the biomass could be preprocessed minimally (i.e., ground) or extensively (pelletized). The depot also provides an opportunity to interface with rail or barge transport if that is an available option. The

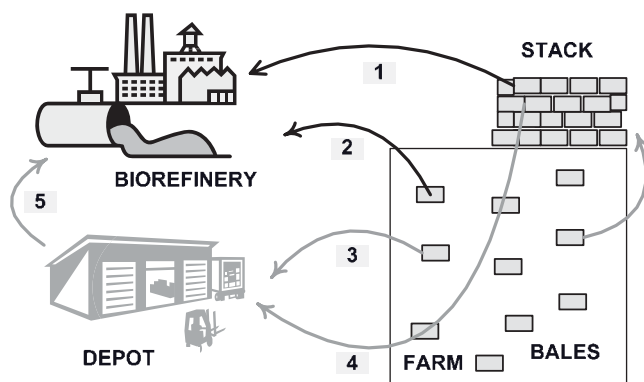


Figure 7.6. Logistics of supplying baled biomass to a biorefinery.

choice of any of the options 1 to 5 depends on the economics and cultural practices. For example, in irrigated areas, there is always space on the farm (in unirrigated corners of the land in a center pivot irrigation scheme) where quantities of biomass can be stacked. In northern dry land farming, the farmer may allow storage of biomass on the field over winter until April but needs the land to seed the new crop.

Economics, Energy Input, and Carbon Emissions

The Integrated Biomass Supply Analysis and Logistics (IBSAL) model was used to calculate cost and energy inputs for the supply chain of biomass (Sokhansanj et al. 2006). IBSAL consists of different sub-modules for harvesting, processing, preprocessing (grinding), storage, and transportation. Model input data include local weather data, average net yield of biomass, crop harvest progress data (including start and end dates of harvest), dry matter loss with time in storage, moisture content of plant at the time of harvest, operating parameters of equipment, and \$/hour cost of machinery. The model is built on the EXTEND™ (Imaginethat, Inc., San Jose, CA) platform (<http://www.imaginethatinc.com>). Main outputs of the model include delivered cost of biomass (\$/t), carbon emission (kg of C per t), and energy consumption (GJ/t). IBSAL also calculates dry matter losses of biomass using limited data available for storing switchgrass bales (Sanderson et al. 1997) and handling hay (Rees 1982). Details of the model can be found in Sokhansanj et al. (2006, 2008).

The choice of particular size and operating conditions are based on three objectives: (1) the latest model of equipment that are commercially available for forage harvest; (2) the typical operational performance data that are available given by the American Society of Agricultural Engineering (ASAE, which is now the American Society of Agricultural and Biological Engineering [ASABE]) standard on Agricultural Machinery Management Data, ASAE D497 (ASAE 2004) or from manufacturer's literature; and (3) limited equipment performance data published for switchgrass elsewhere. Hourly costs are calculated using the procedure and data described in Sokhansanj and Turhollow (2002). The rates represent the sum of fixed and variable costs. The hourly rates for the pull-type equipment (e.g., baler) are the sum of the hourly rate for the implement and the power equipment (e.g., tractor).

Collection Cost

Square baling cost is the highest at \$23.72/t followed by loafing at \$19.21/t (Sokhansanj and Turhollow 2002). The low collection cost using loafer is because of its reduced number of operations and the size of the loaf. The higher cost for dry chopping and piling (\$35.17/t) and for ensiling (\$35.75/t) is because of the higher cost of the forage chopper. Mowing and raking operations are eliminated in silaging operation but the extra cost of pit and packing the silage offsets the lower cost of harvest. The input data for silaging also includes the cost of silage pit at \$4757 per year.

The energy inputs range from 0.319 GJ/dry t for loafing to 0.590 GJ/dry t for the dry chop system. The energy inputs are dependent on the size of power used to operate the equipment. Forage choppers require large amounts of power—more than 200 kW. Using 16 GJ/dry t as the energy content of dry switchgrass, the energy input to the system ranges from roughly 2% for loafing to less than 4% for dry chopping. The energy expenditure for silaging is slightly less than for dry chopping. Conrado et al. (2005) analyzed switchgrass collection and

handling with various types of equipment and concluded that once optimized for switchgrass loafing can become the most cost-effective option.

Preprocessing Costs

Pelletization is the only preprocessing step considered in the biomass supply system. The base case pellet plant has a production capacity of 6t/hour with the annual production of 45,000t (Sokhansanj et al. 1999). The plant operates 24 hours for 310 days annually (annual utilization period 85%). Table 7.4 summarizes the cost of pellet production including variable costs using the system. For the base case, wood shavings at 10% (w.b.) moisture content was considered as a burner fuel with a fuel cost of \$40/t delivered to the pelleting plant. Cost of wood shavings is considerably high because of the high demand for animal bedding materials and as a fuel for the pulp mills. The capital and operating cost of producing biomass pellets are \$5.64 and \$25.18/t of pellet production, respectively. The cost of producing pellets (\$30.83/t) may be further reduced if the plant capacity is increased. Sokhansanj and Turhollow (2004) calculated a cost for cubing of corn stover at \$26.17/t using corn stover as source of heat in the biomass dryer.

For the energy inputs to produce pellets, a sum of 0.821 GJ/t is calculated for the entire process. The sum is roughly 5% of the 16 GJ energy content in a ton of dry switchgrass. The most energy-consuming operation is the dryer (assumed drying from 50% to 10% moisture content), which constitutes more than 40% of the entire energy used for pelleting. Next in the list is the pelleting process followed by the grinder.

There are a number of means of lowering pellet costs and energy consumption. It is possible to move the grinding operation to the field and grind to a bulk density as high as 128 kg/m³. This change in the process sequence would reduce the cost of transporting loose stover and give almost the same density as a bale without the baling cost. Costs might be lowered by as much as \$10/t. Operating the pelleting facility 300 days instead of 240 days/year will reduce costs. Achieving a higher density cube and higher pellet mill throughput, as with alfalfa, would also contribute to lowering costs. Other additional opportunities to reduce costs

Table 7.4. Cost of biomass pellet production for the base case (2004 U.S. dollars).

Pellet Process Operations	Capital Cost (\$/t)	Operating Cost (\$/t)	Total Cost (\$/t)	Energy Use (GJ/t)
Drying operation	2.46	7.84	10.30	0.350
Hammer mill	0.25	0.70	0.95	0.100
Pellet mill	1.43	1.88	3.31	0.268
Pellet cooler	0.13	0.21	0.34	0.013
Screening	0.11	0.05	0.16	0.006
Packing	0.56	1.37	1.93	0.006
Pellet storage	0.07	0.01	0.08	0.026
Miscellaneous equipment	0.42	0.33	0.76	0.052
Personnel cost	0.00	12.74	12.74	—
Land use and building	0.21	0.05	0.26	—
Total cost ^a	5.64	25.18	30.83	0.821
	3.18	17.34	20.53	0.471

^aFirst row of total cost includes drying. Second row of total cost does not include drying.

would include having multiple feedstocks that are available as a fresh supply for as much as 180 to 240 days of the year.

Transport Costs

Transport costs in IBSAL are calculated based upon specifying either a fixed distance or a variable distance. Fixed distance is the transport cost from particular satellite storage (or stacks) to the biorefinery. For example, 5000t of biomass is transported from the satellite storage (or depot) A to the biorefinery. The variable distance scenario is when we specify a total quantity of biomass to be collected from locations within a specified radius (or a minimum to maximum distance). For example, 5000t (or any quantity) of biomass has to be supplied to a biorefinery from within the circle (supply radius). The biomass is supplied from locations A (maximum distance) or any location B or C closer to the biorefinery.

The cost of transporting baled biomass for a variable distance of 20–100 km was calculated by Sokhansanj et al. (2006). The cost of transporting biomass for a fixed distance was also calculated. For transport analysis, the large square bales were loaded on a flat bed (36 bales). The bales are transported to the biorefinery where they are stacked. The bales are ground for entering the process line at the biorefinery. The cost of transporting a maximum fixed distance is higher than the cost of transporting a variable distance between the biorefinery P and storage A.

Transport cost is a strong function of bulk density. Table 7.5 is a list of transport costs for biomass in the form of grind and pellets. Experiments (Mani et al. 2004) with the bulk density of grind size of 2.5 mm shows that a bulk density of 120–180 kg/m³ can be achieved depending on the method of fill and the vibration of the container. We assumed a bulk density of 140 kg/m³ for dry grinds. Pellets can have a density as high as 650 kg/m³. We assumed a bulk density of 580 kg/m³ for pellets. Table 7.5 shows that the grind transport cost is also very dependent on the method of loading. In this analysis we use a front-end loader to load the 100 m³ capacity truck. It is costly at \$9.03/t. Pellets are loaded using the same method but cost only \$2.71/t because of high bulk density. The total cost of biomass transport or pellets for a 20–100-km distance is roughly \$6/t.

Traditional Method of Transport Analysis

The traditional way of handling biomass transport cost is to consider a constant cost component and a variable cost component (Kumar and Sokhansanj 2007) for the transport equip-

Table 7.5. Cost, energy, and emissions for each unit operation in transporting grind and pellets for a variable distance of between 20 and 100 km.

Transport Operations	Grind Transport			Pellet Transport		
	Cost (\$/t)	Energy Input (GJ/t)	Carbon Emission (kg/t)	Cost (\$/t)	Energy Input (GJ/t)	Carbon Emission (kg/t)
Load	9.03	0.395	30.5	2.71	0.118	9.3
Transport	11.51	0.522	40.5	3.27	0.149	11.6
Unload	0.31	0.014	1.1	0.09	0.004	0.3
Total	20.85	0.931	72.1	6.06	0.271	21.2

ment. For truck transport, the constant cost component is the cost of loading and unloading. The variable cost component is the “per km and per t” cost of trucking, accounting for fuel, depreciation, maintenance, and labor. The constant cost in case of rail transport includes the capital cost of rail siding, rail cars, and equipment for loading and unloading biomass. The variable cost includes the charges of the rail company that include capital recovery and maintenance for track and engines and fuel and operating costs. Table 7.6 summarizes the cost of transporting biomass using three modes of transport: truck, rail, and pipeline. The cost equation for pipeline was developed based on the data of Kumar et al. (2004).

Figure 7.7 compares the cost of transporting biomass using three modes of transport. For pipeline the annual capacity is assumed 1 million dry t. In this model, the transport cost in \$/t for truck and rail does not change with capacity (in real situation, the size of contracts with transport companies affects the prices). Pipeline has the steepest cost curve because of the increased capital cost with distance.

Truck and rail costs intersect at about 110 km for the cost figures used in this analysis. It should be mentioned that the cost structures for rail are much more complicated than what is given in this analysis. In cases where a multi-mode transport is required, the cost structures will be a blend of two or three of these modes. At this point we would like to caution against over-generalization of equations in Table 7.6 and graphs in Figure 7.7. The cost of trucking,

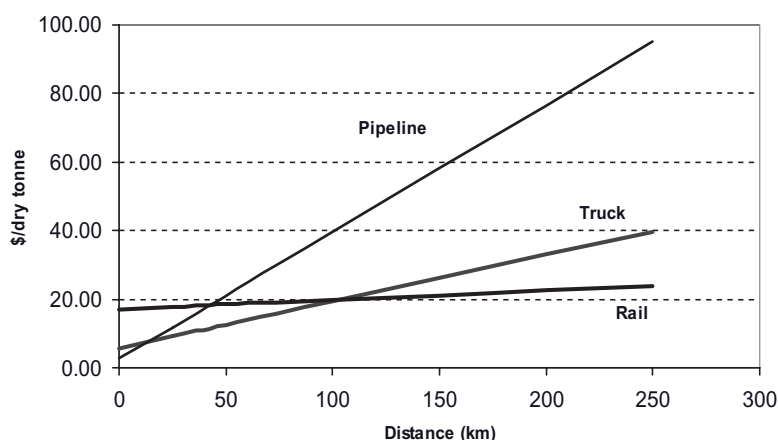


Figure 7.7. Transport cost of biomass using three modes of transport. For pipeline an annual capacity of 1 million t is assumed.

Table 7.6. Cost and energy consumption equations for transporting biomass using truck, rail, or pipeline^a.

Transport Mode	Cost (\$/t)	Energy Consumption (MJ/t)
Truck	$5.70 + 0.1367 L$	$1.3 L$
Rail	$17.10 + 0.0277 L$	$0.68 L$
Pipeline ^a	$2.67 Q^{-0.87} + 0.37 L Q^{-0.44}$	$160.2 Q^{-0.87} + 22.2 L Q^{-0.44}$

^aThe cost and energy values for pipe line are in \$ and in MJ.
L, distance (km); Q, annual supply (million dry t).

Table 7.7. Minimum and maximum cost of biomass supply (20–100 km distance) including granulation (pelleting).

Operations	Low		High	
	Cost (\$/t)	Energy (GJ/t)	Cost (\$/t)	Energy (GJ/t)
Collection	19.69 ^a	0.319	23.72 ^b	0.339
Transport	6.06 ^c	0.271	23.72 ^d	0.339
Granulation (pellet)	20.53 ^e	0.471	30.85 ^f	0.821
Granulation (grind)	5.65	0.096	5.65	0.096
Total	46.28	1.006	78.29	1.509

^aLoafing.^bBaling.^cTransport pellets.^dTransport grind.^eNo drying.^fWith drying.

rail, and even pipeline much depends upon available infrastructure, custom rates, road travel regulations and size of contracts.

Table 7.6 also lists estimates for energy consumption by truck, rail, and pipeline. The energy input for truck and for rail is 1.3 and 0.68 MJ/t/km, respectively (Borjesson 1996; Kumar et al. 2006). The energy input for rail transport is 0.68 MJ/t/km. It is assumed that diesel fuel is used for both truck and rail. The electrical power is assumed to be produced from a coal power plant; we assumed an electricity price of \$0.06/kWh to convert from the cost (\$) to energy (MJ) consumption for the pipeline.

Table 7.7 lists the minimum and maximum costs involved in biomass collection, preprocessing (pelleting), and transport. The delivered cost varies from a minimum of \$46/t to more than \$78/t. This cost does not include payment to farmers that might be around \$10/t. The total energy input to the system ranges from a low of 1 to 1.5 GJ/t. This amount of energy input is roughly from 6% to 10% of the total energy content of biomass (estimated at 16 GJ/t).

Summary

In this section, the state of the art of the existing technologies for supply of biomass from the farm to a biorefinery was analyzed. Several scenarios for potential technologies that will reduce the cost of supply were presented. The analysis shows that the following are key components to reduce costs:

- Reduce the number of passes through the field by amalgamating collection operations.
- Increase the bulk density of biomass.
- Work with reduced moisture content.
- Densification (pelletization or briquetting) is a viable option although the existing technology of densification is expensive.
- Trucking seems to be the most prevailing transport option but other modes of transport such as rail and pipeline may become attractive once the capital costs for these transport modes are reduced.

A biorefinery requires biomass in a form that could yield the maximum conversion products. Among the desirable specifications is cleanliness of the biomass—to be free from dirt, stone, synthetic fibers, and oil. It is also desirable to have biomass at a uniform moisture content and particle size distribution. Further physical and chemical specifications will become important as conversion technologies advance. Biomass also has to be preprocessed to increase its bulk density and its flowability. A densely pelleted biomass takes much less space than a bulky fibrous biomass. The dense pelleted biomass can also flow easily. Biomass can be engineered to meet both the requirement of a biorefinery as well as its low-cost safe handling issues.

Logistics of Biomass Feedstock Handling at the Plant Gate

The inbound logistics and handling of feedstocks at the plant gate is one of the important logistics operations in biomass utilization for food, feed, fuel, and bioproducts. Fuel production or power plants using biomass feedstocks need to have enough inventory to ensure at least 10 days of production, and supply needs to be such that this inventory level is always maintained at the plant gate. Inventory can result in significant costs and operating inefficiencies (Dilworth 1992) and thus careful planning at the production plant must be made to secure the required weekly inventory year round. This planning will include an evaluation of the feedstock material flow layout for receiving operations, storage space, and inventory management to ensure that the quality of stored feedstock is maintained until consumed. Fire hazard prevention is especially important for highly combustible and reactive feedstocks like plant biomass.

The current biorefineries using grains for the production of fuel ethanol were designed to receive the majority of their feedstocks by trucks. Unlike for some other manufacturing operations, feedstocks to be delivered to a processing plant come from hundreds of producers in a region having different travel distances to the plant. The inbound logistics (material flow) of feedstocks delivered at the plant gate should be coordinated such that delivery trucks are able to spend the minimum amount of time waiting to process and off-load their cargo. This will prevent having long lines of waiting trucks at the plant waiting to off-load. Traffic congestion by waiting trucks can pose traffic hazard and also make communities antagonistic to having biorefineries or similar operations in their communities. For example, a 110 million gallon per year (MMGY; 416.4 mil. L per year) corn ethanol plant has about 110 truck deliveries of corn per day with each truck carrying about 25,500 kg (1000 bu) of corn. With a conversion rate of 10.2 L of ethanol per 25.5 kg (1 bushel) of corn, a truck load of 25,500 kg will produce about 10,200 L of ethanol. For comparison with lignocellulosic biomass like corn stover, a truck load of corn stover will hold about 17.5 dry tons of feedstock (39 rectangular bales of 8' length $\times$ 4' width $\times$ 3' height; Mukunda 2007) that can be converted to about 4769 L of ethanol at a conversion rate of 272.5 L of ethanol per dry ton of feedstock. This means that about twice the number of trucks of corn stover (220 trucks) to the plant to keep the same level of production with corn grain are needed in this scenario. Traffic congestion possibilities become a big issue and thus inbound logistics of delivering feedstock to the plant need to be carefully designed to prevent traffic congestion.

This section will discuss the inbound logistics of delivering lignocellulosic biomass to a biorefinery. This will be presented in three sections, namely: (1) a comparison of the inbound logistics of three feedstock types, corn grain, and corn cobs that would give us an idea of

what could be designed for lignocellulosic biomass; (2) components of the inbound logistics of feedstock delivery at the plant gate; and (3) analysis of inbound logistics of biomass delivered to a biorefinery.

Inbound Logistics of Bulk Agricultural Feedstocks

Because of the lack of large commercial utilization of lignocellulosic biomass for liquid fuel production, very little commercial know-how and experience are available to learn from. Therefore, we will examine the inbound logistics of grain and corn cobs in a grain elevator and a dry-grind corn ethanol plant (biorefinery) in order to have an insight of the inbound logistical operations that take place at the plant gate during the delivery of feedstocks for further shipment or the production of bioproducts and biofuels. The brief description of these handling operations is taken from Mukunda (2007) and the material flowchart presented in Figure 7.8.

Grain Handling in a County Elevator

A brief overview of the grain handling in an elevator is presented. There are a number of layouts for inbound receipts of grain trucks, but the one described is from Berruto and Maier (2001). Grain is delivered to a county elevator with hopper bottom trucks with a capacity to carry about 25,500 kg (1000 bushel) grain. The first stop of the grain delivery truck at the elevator is the sampling station where samples of grain are pulled from the truck load using automatic telescopic probes. The pulled samples are graded to determine U.S. grades for grain quality by which the price to be paid is set. The variables tested to determine U.S. grade for corn include the moisture content, test weight (bulk density), broken kernel and foreign material (BCFM) and damaged kernels total (DKT), and heat-damaged kernels (Stored Product Management, E-912 1995). While the samples are being graded, the trucks drive up to an unloading station where it is weighed for gross weight, unloaded in a dump pit, and then weighed again for tare weight to determine the weight of the grain delivered. A ticket is made out for the delivery after this process. The moisture content of the grain determines if the grain is directly sent to one of the many storage silos or if it is first cleaned and dried to a safe moisture level before storage. A discount to the price is estimated for every percent point of moisture removed in drying.

The design layout of an elevator facility is very important and determines the efficiency at which inbound grain trucks flow through the facility, especially when delivering identity-preserved grain or different grain types during peak harvest season. Coordination of the operations in delivering the grain from sampling to exit of the truck using a queue management system is vital to reducing the time it takes to service customers delivering grain to the facility. A goal is to minimize the average waiting time per truck so that the maximum designed capacity for the facility can be obtained for most of its service period. The cost of shipping grain is also reduced if trucks spend a minimal time waiting to receive their cargo. The inbound logistics of grain at an elevator does give us a glimpse of the challenges that would be faced processing large volumes of inbound trucks with plant biomass through a biorefinery.

Cob Handling in a County Elevator

Typically cobs are not handled in country elevators. The system described here is taken from Mukunda (2007) and also based on the authors' visit to this facility. Corn cobs are delivered by trucks from seed processors to the facility where they are weighed and blown

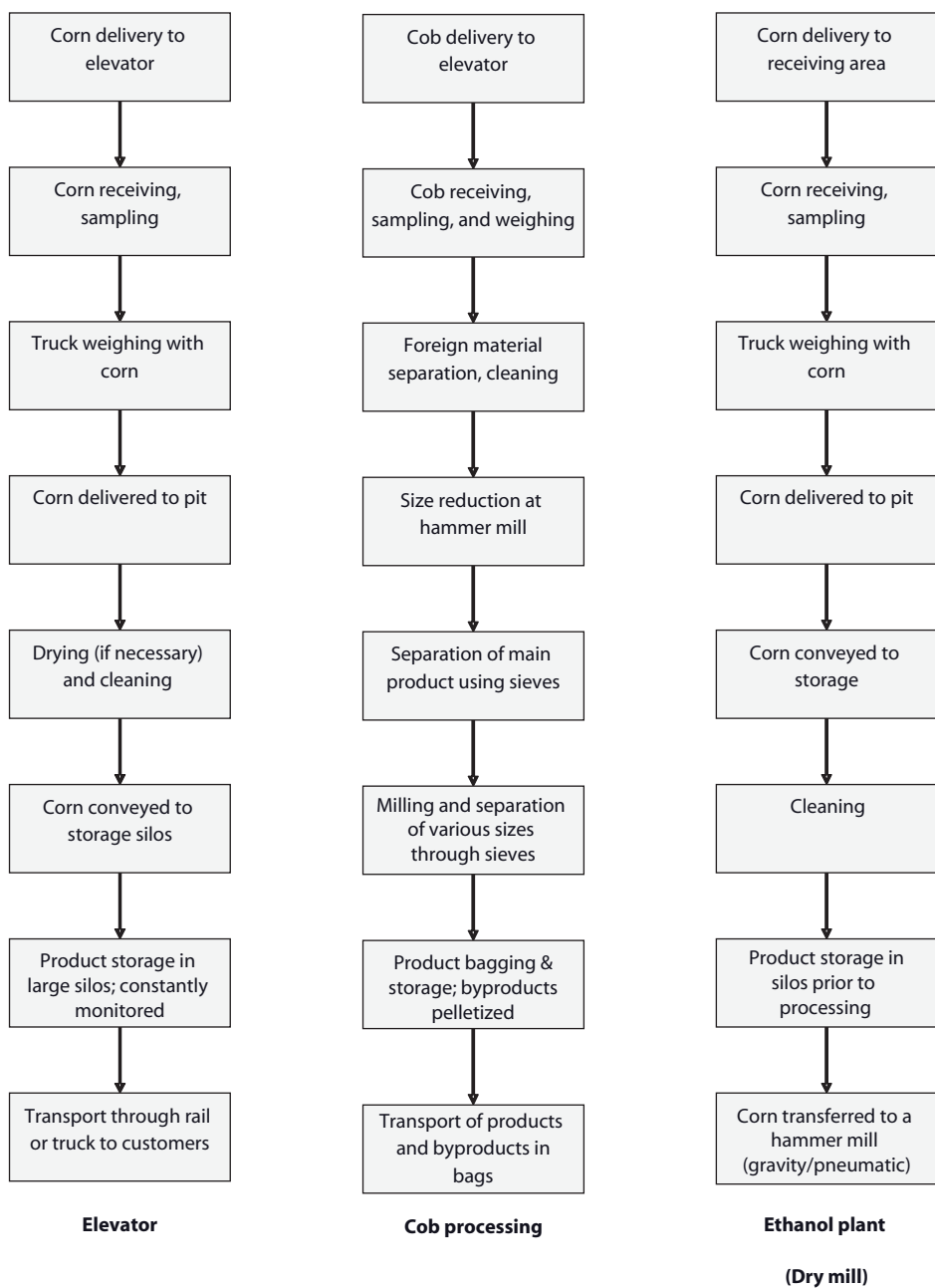


Figure 7.8. Grain and bulk (cob) handling operations in an elevator and in dry-grind corn ethanol plant.

pneumatically unto a pile several meters high. Trucks are weighed before being unloaded but no grading is done to the cargo. The cob pile is stored uncovered and reclaimed daily for processing at the cob mill about 400 m from the cob pile by using front bucket loaders. Cobs delivered to the processing plant are first dried with a rotary drum dryer and stored in steel silos from which they are fed into the plant and processed to various dried granular bulk products that are packaged in 50-lb bags or large 2000-lb bulk bags.

The inbound receipt of cob delivery trucks appear not to be a bottleneck at this facility, because the inventory of cobs at the facility stored in the outdoor pile is large to meet many months of production. Because the topmost layer of cobs on the pile protects the cobs beneath the pile from deterioration above the threshold quality limit for use, the outdoor storage can be used as a low management system in this operation. Outdoor storage can be applied to cobs because of their rigid solid form, which does not drastically deteriorate as a result of spoilage from inclement weather, making handling very difficult. However, spontaneous combustion and fires that smolder for days occur in the cob pile at this plant once every several years. Therefore, the management approach of outdoor storage at this plant, while low in operational cost, can be expensive if all the pile is lost in a fire. Outdoor storage might not be feasible for all biomass types and so the effect of storage on subsequent handling must be considered on a biomass-type basis. For example, the mechanical strength of lignocellulosic plant biomass, like wheat and rice straw or switchgrass, would degrade much faster in outdoor storage than corn cobs, and this would affect their subsequent handling and processing such as grinding and flow through material-handling equipment.

Grain Handling in a Biorefinery

Most corn ethanol dry-grind plants in the Midwest usually keep an inventory of corn for 10 days of production (Mukunda 2007). Corn is normally delivered to the plant by trucks from within a 60-mile radius. Just like in elevators, delivery trucks queue in line to be sampled, weighed, and their cargo dumped. The rule of thumb is that for every 1 MMGY of plant capacity, a truck of 1000 bu (25,500 kg) of grain is delivered daily. Thus, for a 40, 60, and 100 MMGY, an average of 40, 60, and 100 trucks will deliver corn to the plant daily. With the current capacities in fuel ethanol plants, there has been no report about challenges or difficulties in receiving grain. In fact, some of the ethanol plants have contracted their grain procurement to the major grain logistics operations that have many years of experience and infrastructure handling and shipping grain. If these plants were to be retrofitted to process cellulosic feedstocks, how would their current layout designed for granular feedstocks perform? This could be one of the major challenges faced by existing biorefineries using grain feedstocks that are looking to change to cellulose in the nearby future. In the next section, a framework for designing and mapping the layout of a biorefinery or preprocessing facility using lignocellulosic feedstock will be discussed.

Components of an Inbound Logistics of Agricultural Feedstocks

The design of inbound logistics of feedstock delivery must consist of the following three unit operations: a sampling station, an unloading station, and storage space. The design layout and accompanying equipment will depend on the type of feedstock to be processed. For lignocellulosic feedstocks such as plant biomass or woods, the form in which the feedstock is delivered will determine how the layout is designed and what types of equipment will be in the operations.

Feedstock Sampling

Feedstock sampling of bales can be quite challenging as well, especially when receiving large volumes of feedstocks. Because of the lack of commercial production of biofuels for cellulosic biomass feedstocks, automated sampling equipment for bales might not be available. Additionally, it is not certain how feedstock quality would affect conversion performance to fuels for biochemical processes. However, other handling operations such as size reduction and feeding will be affected when their physical properties change due to deterioration. The growth in commercial production of biofuels from cellulosic feedstock and the future trade of biomass as a commodity for energy will determine the value premium processors are willing to pay for a superior-quality feedstock and how the feedstock is graded.

Handling of Baled Feedstock

Lignocellulosic plant biomass like corn stover and switchgrass is harvested and packaged in bales that are delivered in flatbed trucks. Unlike granular feedstocks that have a high density and are free flowing, bales have low density, typically 160–200 kg/m³. They come in bulky packages of biomass that are either round, square, or rectangular, and require man-operated handling equipment like forklifts and telescopic loaders to unload and load them. Weighing stations with weigh bridges for truck commonly available at elevators and ethanol plant can be used for biomass. Weighing stations would need to have the necessary handling equipment such as forklifts to unload bales in a minimum amount of time. However, it must be noted that the logistical cost for handling are higher with bales than with grain, which can use automated handling conveyors. The development of innovative concepts for automatic unloading of bales from trucks and their stacking in a storage building is needed for commercial utilization of biomass on a large scale.

Handling of Preprocessed Feedstock

Preprocessing biomass into bulk particulate solids will increase the feedstock density and thus enable a large quantity of feedstock hauled per truck to the facility. It also enables the use of automated handling equipment for granular feedstock that is commonly available in the industry. Densifying operations have already been discussed extensively in the Biomass preprocessing section and the Operations to produce dense biomass section, and so will not be discussed here. Trucking cost to the plant and inbound logistics cost will be reduced by densification. As was noted before, the cost of densification can be very high. While the economics of densification had looked less favorable under low world petroleum prices and relaxed environmental policy, recent high prices of petroleum fuel and efforts to reduce global carbon emissions make the use of densification a viable option to reduce feedstock delivery cost. Improvements in densification and reductions in cost will make this approach a more viable option than using bales directly.

Storage Space

Having enough storage space to maintain the minimum level of inventory (10 days) is vital to how much a facility can store on site. The quality of space and level of automation of the handling systems can greatly affect feedstock logistics. While some advocate that outdoor storage is adequate for biomass, this statement cannot be generalized as a rule of thumb, as



Figure 7.9. Comparison of (a) indoor storage of biomass feedstock and (b) outdoor storage of biomass feedstock.

was earlier pointed out for the outdoor storage of corn cobs. Poorly stored feedstocks in outdoor storage will biodegrade more rapidly than indoor storage. Biodegradation of feedstock could result in nutrient variability in the feedstock that will negatively affect conversion, handling challenges in conveying and processing, and potential fire and health hazards because of spontaneous combustion and inhalation of mold spores from deteriorating biomass. In general, woody feedstocks are more favorable to outdoor storage than herbaceous biomass. Figure 7.9a,b provides a good pictorial comparison of indoor stored bales of plant biomass and outdoor pile subject to deterioration by inclement weather.

Analysis of the Inbound Logistics of Biomass Delivered to a Biorefinery

The inbound logistics of delivering biomass from the field to a biorefinery has been investigated by the authors (Mukunda et al. 2006; Mukunda 2007). A model named, Biomass Feedstock Logistic Simulator (BmFLS) using discrete event simulation was developed on EXTEND™. The model consisted of four blocks that simulated the following: (1) feedstock generation from the field at harvest; (2) feedstock storage and loading at the field; (3) transportation to the biorefinery; and (4) inbound logistics operations at the biorefinery.

The analysis discussed here would only pertain to the feedstock inbound logistics. In developing a scenario for the analysis, an existing 102MMGY corn ethanol fuel plant located in Indiana was assumed to be supplied corn stover feedstock. The inventory to be maintained was for 10 days of production. A conversion rate of 72 gallons/dry ton of corn stover was used and 900lbs of $8' \times 4' \times 3'$ was assumed at the bale weight delivered to the plant from seven different farms that were categorized based on their sizes. These farms ranged from 10 to 80 miles of supply radius to the biorefinery and corn acreage data were taken from the National Agricultural Statistics Service (NASS) to compute the feedstocks collected from these radii mileage. Figure 7.10 (Mukunda 2007) shows the percentage of feedstock at various distances from the plant. It is important to observe that feedstock will be delivered from farms located at a range of mileage. This needs to be factored in when analyzing the travel times and arrival distances of trucks to the plant.

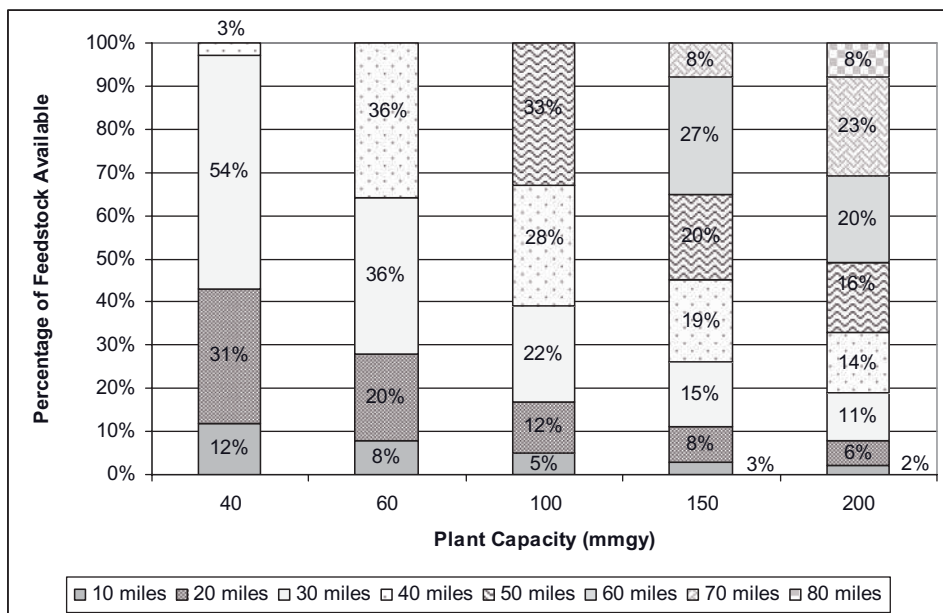


Figure 7.10. Percentage of feedstock from different distances for different plant sizes (Mukunda 2007).

One of the first goals to tackle in analyzing the inbound logistics is to determine the frequency of feedstock delivery needed for maintaining the inventory level at the biorefinery. Feedstock delivery will have some restrictions based on the working hours of the plant delivery schedule, the number of trucks that can be processed through, and the plant sampling, and unloading stations. The simulation tracked inventory at the plant and truck queuing inside the plant (Mukunda 2007) and the following statistics were collected: average feedstock inventory, total trip time, total service time, waiting time and utilization of the weighing, sampling, and unloading stations.

As mentioned before, the number of delivery trucks needs to match the sampling and unloading station capacity at the plant for trucks to be serviced within the limit of service hours (8–16 hours/day). Station utilization (sampling, weighing, and unloading) must be optimized to reduce redundancy. The number of stations needed is dictated by the numbers of inbound truck deliveries to the biorefinery and there is a trade-off between the number of trucks and unloading station capacity (Figure 7.11; Mukunda 2007). The total trip time, service time, and waiting time all depends on the size of the biorefinery. On average, all these times increase with increasing plant sizes as shown by the analyses of plants capacities from 40 to 200 (Figure 7.11; Mukunda 2007). Reducing these times will reduce the cost of feedstock delivered.

As mentioned previously, the trucking cost is by far the most expensive of all the logistics components. Analysis conducted for 100MMGY showed that the trucking cost to the plant over 10 years can be 30 to 40 times more than the handling cost at the plant (Mukunda 2007). This means more emphasis should be placed in optimizing transportation tonnage in order to have a real impact on the logistics of handling bulky plant biomass feedstocks.

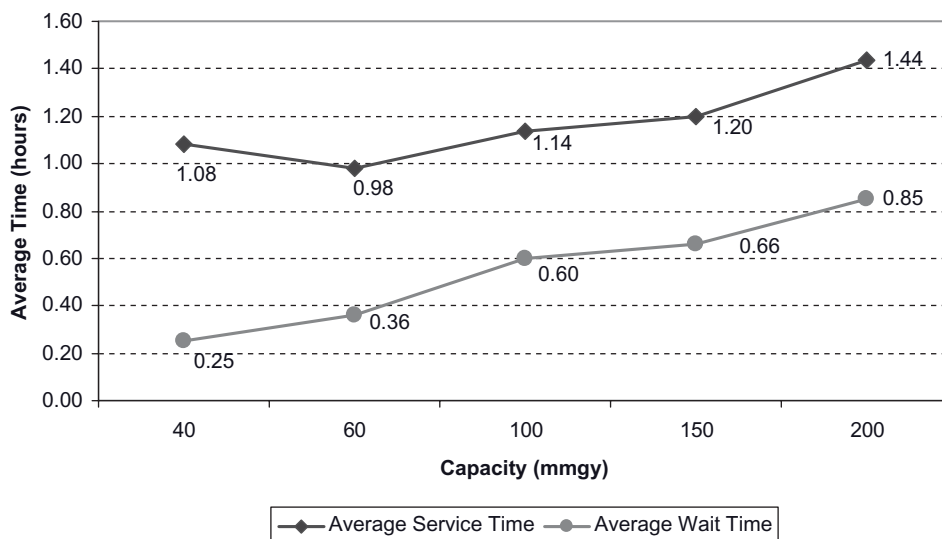


Figure 7.11. Average service time and wait time for trucks supplying plants of various capacities (Mukunda 2007).

Related Agricultural Logistics Operations and Their Applications to Biomass

This section discusses existing commercial logistics operations of agricultural feedstocks that are similar to lignocellulosic biomass feedstocks. The principles required for an efficient logistic system for biomass are illustrated by several commercial operations. Two examples of the collection of herbaceous fiber are explained in detail, the cotton harvest throughout the Southern United States, and the sugarcane harvest in South Florida. Throughout the discussion on the cotton and sugarcane systems, the reader will be reminded of certain features of each system that relate to the design of a logistic system to deliver herbaceous biomass to a bioenergy plant.

The sugarcane harvest in South Florida illustrates an efficient system for a high-yield, high-moisture content crop and is a commercial example for tropical grasses with high yield, (55–125 t/ha) and high moisture content (80%). On the other end of the spectrum, the cotton harvest is the commercial example for a lower yield (2–4.5 t/ha) and lower moisture content (20%) crop.

The largest harvest of biomass in the United States is the wood harvest for the forest products industry. A byproduct of the harvest of logs for this industry is the harvest of “fuel chips.” This harvest is a major commercial example of a biomass harvest for bioenergy, thus it is an important reference point for our discussion of herbaceous biomass logistics. Anyone seeking to design a herbaceous biomass logistic system should first look at the fuel chip industry and learn the “tricks of the trade.”

The two herbaceous fiber examples, cotton and sugarcane, are contrasted with a grain example, the corn harvest in the U.S. Midwest. Grain is a flowable material, and the system for handling, storage, and transport is mature technology that was simply “plugged into place”

for the corn ethanol industry. A fiber material is not flowable, certainly not to the degree grain is flowable, thus the material handling is more difficult, and the solutions are more costly. Although the technologies are not directly transferable, it is instructive to compare the performance parameters for the grain logistics system with the systems for handling herbaceous biomass as fiber.

Parameters compared for the three examples are (1) capacity of harvesting unit (t/hour); (2) highway hauling equipment; (3) location of storage; and (4) operation of a receiving facility at the processing plant. A harvesting unit is defined as a harvester and the infield hauling equipment required to keep it operating. Typical productivity and cost parameters are included to facilitate comparison between the three systems.

Cotton Harvesting and Logistics

Harvesting

Cotton is harvested by removing the fiber from the other plant parts. Two different harvester designs are used. These machines are representative of a machine that collects material, separates the desired portion, and drops the rest back onto the ground in the field. Grain combines, of course, have the same functionality.

The spindle harvester has a picking head with a series of cone-shaped spindles with rows of serrated ridges parallel to the axis. These spindles rotate and wind the cotton fiber around the spindle, thus pulling it from the bole. The picking head rotates as the harvester moves forward and doffers sweep the cotton from the rows of spindles. The cotton is then pneumatically conveyed to the storage basket on the harvester.

The stripper harvester has a mechanism to aggressively comb through the standing cotton stalks and collect the fiber. It tends to collect more plant parts (pieces of stem, leaf, and outer hull of the bole) than the spindle harvester.

Raw cotton collected from the field includes the seed, therefore it is referred to as “seed cotton.” A cotton harvester is actually a mobile solid–solid separator technology. The seed cotton (a solid) is separated from the other plant parts (solids).

Infield hauling is done with side-dump wagons sometimes referred to as “bole buggies.” When the harvester basket is full, the harvester stops, the side-dump wagon pulls alongside, and the basket is dumped (Figure 7.12). The wagon then proceeds to a location where the module builder is parked, typically close to a public road to provide ready access for the module hauler.

The wagons cycle continuously between the harvester and module builder until the harvest of a particular field is complete. Harvesting cost (\$/t) is lowest when harvester wait time is minimized. Ideally, the module-building location is chosen to minimize cycle time of the wagons. However, in reality, it must be chosen such that road trucks can get into position to pick up the modules without getting stuck, thus there is a trade-off between wagon cycle efficiency and road truck access.

Logistics

The cotton module is an 8-t block of cotton (Figure 7.13) that serves as a “farm-level” storage for seed cotton. It is covered with a canvas or plastic cover and can stay in position for several weeks, if needed, before being hauled to the gin.



Figure 7.12. Cotton harvester dumping into side-dump wagon used for infield hauling of cotton. Side-dump wagon subsequently dumps into a module builder at the edge of the field.



Figure 7.13. A module hauler loads a covered cotton module stored at edge of field with convenient access for road trucks.

The roadside storage option provided by the cotton system has been incorporated in one concept for a logistics system to supply a bioenergy plant. *It is appropriate to simply highlight at this point that the disconnect between the infield hauling and the highway hauling operations is a significant advantage of the cotton system.*

Module haulers are special trucks that are only used to haul cotton modules. The hauler shown in Figure 7.13 has a set of rubber tracks mounted at the rear and powered with a driveline from the truck transmission. The bed of the truck is tilted until the tracks engage the ground, then the tracks pull the truck (drive transmission in neutral) under the module.

A set of chains on the floor of the bed moves the module up into the truck. It is not possible to “pull” the module up into the truck, thus the chains are synchronized with the tracks such that the truck moves rearward at the same rate the module moves up the floor of the bed. Typically, the entire 8-t module is loaded in about 5 minutes. Rapid load time is the advantage gained with the module hauler; the fact that these trucks are only used during ginning season is a disadvantage.

At the gin, the process is reversed to set the module back on the ground in the gin storage yard. When this module is needed, a module hauler picks it up and places it on the conveyor that feeds modules into the gin. The module feeder has a series of beaters (bars with spikes) that rotate and “eat away” the front of the module as it moves forward, thus establishing a continuous flow of cotton into the gin. The logistics system has not completed its function until it has established a continuous flow of raw material into the plant. *Delivery of loads to the plant is not a terminal point.*

Some cotton gins transport all modules from the farms and completely fill the gin storage yard before beginning the ginning season. This procedure requires a large graveled storage yard. Most gins operate a smaller storage yard and haul modules throughout the ginning season. The ginning season ranges from 70 days in Virginia to 120 days in Arizona.

Cotton Gin Operation

A cotton gin is basically a very sophisticated solid–solid separation technology. It not only removes the fiber from the seed, but also cleans the plant material from the fiber. The fiber is then baled into 220-kg bales that are enclosed in a plastic sleeve for protection. The bales are then stored in a warehouse at the gin and are shipped in tractor-trailer trucks to meet the demand schedule of the cotton buyer, typically a textile mill.

A cotton gin produces three main products:

1. Cotton fiber (bales)
2. Mote cotton (short fiber removed from seed and used for a variety of industrial applications)
3. Cotton seed (an important ingredient in dairy cattle feed)

The remaining material is referred to as cotton gin waste (CGW). Some of this material is fed to beef cattle, some is composted, and some is land applied. There are continuing efforts to find higher value uses for CGW, and some of these efforts may lead to its use to produce a biofuel.

The cotton gin not only stores the bales for continuous delivery to the textile industry, but it also stores seed for year-round delivery to the feed market. In this sense it functions as a preprocessing plant in the total logistic system—field to factory. *The function of a preprocessing plant is to receive raw material and produce a more homogeneous product, or products, which meet customer quality standards and can be shipped more cost competitively to meet a year-round delivery schedule.* This is the job of the cotton gin.

The cotton gin is an interesting model for the type of preprocessing plant that might be included in a biomass system. The raw biomass is brought in from the field and processed into products sold to three different markets. After ginning the products have at least twice the value (\$/kg) as the raw biomass. This increase provides an opportunity to ship longer distances (because a truckload has a higher value), a benefit that could potentially be realized with a preprocessing plant in a biomass system. Raw biomass can be bulky, and thus has a

low energy density per unit volume. If it can be transformed into a material with higher energy density, a point made earlier in this chapter, then this material can be hauled further to support a larger processing plant.

There is another lesson to be learned from the cotton industry. There is a compromise between the level of separation done with the mobile machine (cotton harvester) and the stationary machine (gin). In general, the annual hours of operation for a mobile machine is limited by field conditions (condition of crop, weather, and daylight) and these machines require a liquid fuel (diesel). Stationary installations can operate 24/7 and use electric power, thus avoiding, at least until electric cars become popular, a direct competition with the transportation sector for energy. *System cost (harvest plus logistics) is often minimized when the mobile machine is simplified and more processing steps are allocated to the stationary machine.* This is certainly the case with the cotton system. No one has advocated that the cotton harvester be modified to accomplish the ginning function and thus become a “mobile gin.”

Sugarcane Harvesting and Logistics

Sugarcane is a high-yield (up to 155 t/ha), high-moisture content (80+%) crop. It is grown to collect the sugar (sucrose) produced in the stalk. The stalks are crushed and the juice collected. This juice is then concentrated into molasses that is subsequently centrifuged to produce crystalline sugar. The raw sugar is washed to produce the pure white product we see in the sugar bowl on our breakfast table.

Harvesting

A sugarcane harvester cuts the stalk at the base, and then cuts it into 30-cm-long billets. Air is blown through the billets as they are cut to blow away as much leaf as possible and leave it in the field. The billets are conveyed into a side-dump wagon that travels with the harvester. There is no onboard storage on the sugarcane harvester, as with the cotton harvester, thus the side-dump wagon must be in place for the harvester to operate.

The side-dump wagons proceed to the edge of the field where an elevated loading ramp is prepared (Figure 7.14). The wagons dump directly into bins on trucks, and these trucks deliver the bins directly to the sugar mill.

Logistics

Cycling of the side-dump wagons and the highway trucks must be well coordinated for the harvest to proceed with maximum efficiency. The cut stalks spoil so quickly that there is no storage between field and mill. *This same constraint will apply to any option that proposes to ferment a “sugar biomass” directly to produce ethanol.*

Truck trailers hauling the bins are equipped with a pivot point such that the bins can be dumped directly onto the conveyor feeding material into the mill. Time to dump a load (trailer with two bins) is 3 minutes. Some truck tractors pull a double trailer (four bins), and the time to dump this load is 7 minutes. If a given load is not needed to fill the conveyor, the loaded bins are removed and stacked two high on a graveled storage yard. Empty bins are removed from storage and placed on the truck to be returned to the field. This operation takes 3–4 minutes. One sugar mill in South Florida unloads 1000 trucks per day, a total delivery of about 24,500 t/day.



Figure 7.14. Side-dump wagons loading trucks at the edge of sugarcane field.

Harvesting and hauling is done only during daylight hours, thus the at-plant storage is essential to operate the plant 24/7. Each bin has a pivot point so it can be dumped during the evening when placed in position with the forklift. The sugarcane system is unique in that harvesting and hauling does occur 7 days per week.

One sugar company in South Florida harvests and processes 3.3 million t of sugarcane in a 140-day season. The industry is a unique example of plantation agriculture. The company owns the production fields, the harvest equipment, the service roads through the fields, and the sugar mill. It owns the trailers that haul the bins, but typically contracts with trucking companies for the truck tractors that pull the trailers.

When the truck tractors pull a double trailer, the second trailer is hooked behind the main trailer. Each trailer hauls two bins for a total of four bins, thus the total length of the vehicle is almost 35 m. A vehicle like this would not be legal on most public roads across the United States. The labor productivity (t/hour) for truck drivers in the sugar industry cannot be equaled for any other biomass logistics.

Trucks typically haul 10 loads per day. With no delays, they could theoretically haul 13 loads per day, thus the truck productivity factor is $10/13 = 0.77$, or 77%. This exceptional performance is possible because one entity, the sugar company, has control of all segments of the harvest logistics system. The harvest equipment is managed and trucks are scheduled to minimize the load and unload times. This management capability is key to the efficiency of a short-haul operation. It is also significant that the trucks travel on company-owned roads, thus they do not encounter the traffic seen on public roads. It is unlikely that any other biomass system that collects material from fields and delivers it to a central plant can equal the truck productivity, 77%, of the sugar industry in South Florida. Also, it is unlikely that the level of coordination between infield hauling and over-the-road hauling can be duplicated anywhere else. It certainly cannot be duplicated if farmers haul in the raw biomass and make deliveries on their own schedule.

Another sugar mill in South Florida uses a “mobile accumulator” concept to supply the mill 24/7. This mill has railroad tracks extending from the mill out through the production

fields. There are loading platforms at periodic intervals along these tracks. Side-dump wagons fill the railcars at the loading platforms. When the cars are filled, the train is pulled onto a siding at the mill. As needed for 24/7 operations, the cars are moved into position and dumped.

Again, the plantation model offers an advantage. The sugar company owns the railroad tracks through their production fields, thus they can use railcars as a mobile storage for the sugar mill. Once the investment in tracks and other infrastructure is recovered, the train can be more economical than trucks hauling bins.

Fuel Chip Harvest and Logistics

The residues left in the forest as a byproduct of a timber harvest are often chipped and sold as a boiler fuel. This material is referred to as “fuel chips.” Whole trees are cut and brought to a preprocessing location in the woods known as a “landing.” Here the tree is de-limbed and sawed into logs of various lengths. The logs are loaded onto trucks for delivery to a sawmill (or pulp mill) and everything else, limbs and tops, is put through a chipper and blown into a chip van. If the logging contract calls for a clear-cut, then any non-merchantable trees are also brought to the landing and these trees are put through the chipper.

Some landings are *mobile* preprocessing plants. The chipper is mounted on a trailer so that it can be readily relocated to harvest a different tract of timber. Loggers try to optimize the in-forest transport of the raw biomass (skidding of whole trees along the ground) relative to the hauling of product (logs and chips) in highway trucks. The quicker they can get the material off the ground and onto a truck, the lower the total transport cost from stump to final use.

In the past, fuel chips have been sold at a price that basically just covers the cost of chipping and hauling, and perhaps a small part of the cost to bring the trees to the landing. The land owner gets his or her portion of the price for the logs but nothing for the fuel chips. The advantage to the landowner is that the site is cleared and ready for replanting when the logger leaves. The logger gets an advantage because the litter (limbs and tops) at the landing is cleared away and does not accumulate to slow operations. The fuel chip market, however, is changing rapidly as new bioenergy options compete for the chips, particularly in the Southeastern United States.

When there are no delays, a chip van can be filled in about 40 minutes. (High performance chippers are available that can fill a van in 15 minutes.) Average productivity is considerably less in a typical operation. Often, the hauler unhooks and leaves an empty van to be filled while delivering a full van. Waiting for the van to be loaded reduces the number of loads a truck tractor can pull in a given day.

The largest wood-fired electric-generating plant east of the Mississippi is an 80-MW plant in Hurt, VA. This plant unloads 150 chip vans on a typical day; their record is 311 vans in one 24-hour period. The trucks are weighed in, dumped by the truck driver, and weighed out. Total time required when there is no queue is about 10 minutes. *For any bioenergy plant receiving fuel chips, the key to controlling hauling cost is the operation at the receiving facility. This was discussed previously in “Logistics of biomass feedstock handling at the plant gate” section. Haulers do not like to wait in the queue.*

In the Southeastern United States, woody biomass is harvested year-round—it is stored in the forest until needed. Because just-in-time delivery is not practical, some at-plant storage is required. A host of operating variables (weather, traffic, equipment breakdowns) can interrupt deliveries. The 80-MW plant operates with about 20 days of at-plant storage in the summer and 45 days in the winter, when the potential for weather delays is greater. The

biomass is piled with two bulldozers that operate during the daytime delivery. These bulldozers push material onto the storage pile and also push material into a twin-screw feedbox that meters material onto the belt conveyor into the plant. This conveyor must run continuously to maintain a flow of fuel into the boilers. The cost penalty for shutdown is very high. One bulldozer operates during the night shift to keep the conveyor feedbox continuously supplied.

Comparison between Commercial Herbaceous Systems and Fuel Chip System

There are two principles common to the logistic system for the herbaceous biomass examples (cotton, sugarcane), and the woody biomass example (fuel chips).

1. Hauling efficiency (t-per-day-per-truck) is maximized by procedures that minimize the truck load time and unload time. This principle is the key to any short-haul logistics system.
2. Some at-plant storage is unavoidable, thus a system must be in place to facilitate the flow of material into and out of at-plant storage. The sugarcane harvest operates closer to just-in-time delivery than the other two harvests, because it is an integrated system under one management. Even the sugarcane system, however, requires some minimum at-plant storage to provide for uninterrupted operation during the night hours.

An advantage is gained when the infield hauling and over-the-road hauling operations are uncoupled, as is done with the cotton system (Figure 7.15). An additional advantage is gained when the harvesting operation is uncoupled from the infield hauling. Two new cotton harvesters have recently been introduced. One bales the seed cotton in a block about half the size of a conventional module, a concept that emulates the big square bale in hay harvesting. The other concept bales the seed cotton into a 2.4-m diameter round bale and wraps it with a solid plastic sheet. Both these concepts were developed to uncouple the harvesting operation from the infield hauling. With this uncoupling, the harvester can proceed without having to wait for the infield hauling. In fact, the hauling can be done several days, or perhaps several weeks, later.

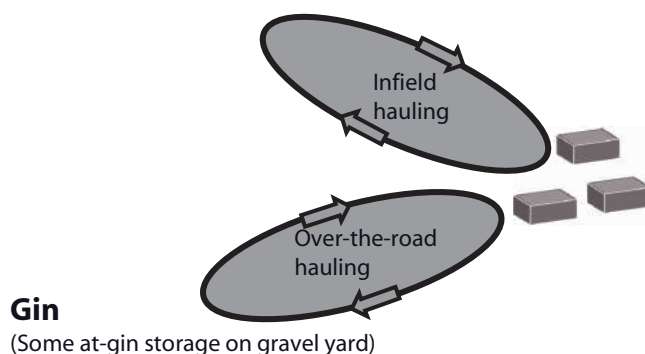


Figure 7.15. Logistic “chain” for cotton harvest. Note the uncoupling between the infield hauling and the over-the-road hauling.

Fuel chips are a flowable material. Size reduction is done in the forest, and the flowability advantage is then realized throughout the remainder of the logistics system. The truck can be unloaded by flowing the material out of the back. When there is no queue, unloading (total time at the plant) is 10 minutes. The use of the bulldozers to flow the fuel chips into and out of at-plant storage is expensive, but study has shown that it is a least cost option; the estimated cost to operate the at-plant storage is less than \$2/t. This cost is increasing as diesel fuel costs increase, thus other options may become competitive in the future.

One option for a herbaceous biomass system is to emulate the fuel chip system and do the size reduction in the field. Another option is to remove biomass from storage (woody biomass can be harvested year-round but herbaceous has a limited harvest season, thus some storage is unavoidable) and chop the material and blow it into a van trailer, as is done with the fuel chips. Can chopped grass be dumped out of the back of a van trailer after it has been vibrated during transport? Also, a plan must be put in place to flow the chopped material into and out of at-plant storage, and the question remains—can this be done in a cost-effective manner?

The need for some minimum at-plant storage is acknowledged by most bioenergy plant design teams. The alternative is just-in-time delivery from field/forest for 24/7 operation, and this is not judged to be a practical option.

It is appropriate to review the two at-plant storage options currently in place. In the cotton system the modules are stored on the ground in a graveled area at the gin. When a module is needed, the “yard” module hauler picks up a module and places it in the conveyor that feeds the module into the module feeder, which meters a continuous flow of seed cotton into the gin. The sugarcane system is similar to the cotton system—the raw biomass remains in the hauling “package” until it is processed. In this case, the hauling bins are stacked two high in a graveled lot at the mill and dumped as needed throughout the night shift to maintain a continuous supply of material into the mill.

Comparison between Herbaceous Fiber and Grain Systems

Harvesting

There are many different models and sizes of combines for harvesting grain and several different models and sizes of cotton harvesters. In like manner, there are a number of different machines for harvesting sugarcane. The manufacturers of harvest machines all state that the capacity of their machine (t/hour), averaged over a day’s operation, is dependent on the infield hauling operation, and, in the case of grain and sugarcane, it is also dependent on the cycling of the highway hauling trucks. This is a key point when the performance of a system of equipment is simulated. Capacity of a “middle” unit in the system depends on the “upstream” unit and the “downstream” unit. Often an advantage can be obtained by uncoupling the harvest operation from the infield hauling operation (sometimes not possible) and by uncoupling the highway hauling from the infield hauling (sometimes not possible). Let us see how this works when we compare the grain, cotton, and sugarcane systems.

A grain combine has an onboard grain tank. When this tank is full, an infield hauling unit (truck, trailer, or wagon) must come alongside so the grain can be unloaded or the combine must stop and wait. An obvious point must be emphasized. Capacity of the most sophisticated (and expensive) combine is 0 t/hour during the period when it is not moving.

The cotton harvester also has some onboard storage capacity. When this chamber is full, a side-dump trailer must come alongside to receive the seed cotton, or the harvester must stop and wait, thus reducing its average capacity (t/hour).

The sugarcane harvester is an even more dramatic example of the linkage between harvesting and infield hauling. The sugarcane harvester, like a forage harvester, has no onboard storage. An infield hauling unit must be alongside to catch the harvested material or the harvest operation stops. Cycling of the infield hauling units is the key to sugarcane harvesting. Remember that the infield hauling units must be able to dump at the truck loading station in order to cycle back to the harvester. Now there is a direct coupling of the infield hauling and the highway hauling. When all operations are under central control, like with the sugar mill, an inter-connected logistic system like this can be the most efficient option. Unfortunately, there are few locations, other than the sugar industry (Florida, Louisiana, Texas), where it is practical.

It is appropriate to define a “harvesting unit.” This unit is defined as one (or more) harvester(s) and the pieces of equipment needed to haul material at a rate that keeps the harvester(s) moving. Excess infield hauling equipment and over-the-road equipment (each unit spends time waiting for the harvester) is generally not the most cost-effective organization of the unit. Trade-offs are always used in organizing a unit. In a commercial operation, each piece of equipment will spend some time waiting—scheduling is never perfect. Because the harvester costs more to operate per hour, it is generally best to minimize harvester wait time.

The harvest units described below are given to provide a reference point. They are not offered as an optimum solution.

Infield Hauling

The grain harvest unit is assumed to be harvesting a field located 20 km from a grain storage. Reasonable combine efficiency will require two operators on infield hauling units and two truck drivers hauling with tractor-trailer trucks to the grain storage. Total crew required is then 1 (combine) + 2 (grain wagons) + 2 (truck drivers) = 5.

To achieve reasonable cotton harvester efficiency, the unit will require the following crew: 1 (harvester) + 2 (side-dump wagons) + 1 (operator for module builder) = 4. As compared with the grain operation, the crew size is reduced from five to four by disconnecting the field operations from the highway hauling. Remember, the cotton module is covered and left in roadside storage to be hauled later. Infield hauling is not delayed when a truck is not present to receive the seed cotton. Another key difference between the grain and cotton systems is that the highway hauling is done by the gin, not the farmer/producer.

A sugarcane harvesting unit is defined as 5 harvesters, 8 tractors pulling 3 wagons each for a total of 24 wagons, and 1 operator at the ramp for a total crew of 14. If the field is only 3.5 km from the sugar mill, the over-the-road hauling will require eight tractor-trailer trucks each hauling two bins. If haul distance is 15 km, then 15–18 trucks are required. At 40 km, it requires 25–28 trucks to keep the harvest unit moving.

Capacity for a harvest unit with good harvest management is given in Table 7.8. There are many production situations where the average capacity for an entire day's operation will be only half the capacity shown in Table 7.8. As a reference point, the cost is also shown. These cost numbers are constantly changing, thus they should not be used out of context.

Hauling

Truck tractors used for over-the-road hauling are a mature technology. (There are over 250,000 trucking companies in the United States, and truck manufacturers work continuously

Table 7.8. Harvest unit capacity and cost for three commercial herbaceous biomass systems. (Cost does not include over-the-road hauling.)

System	Capacity		Cost	
	mton/hour	dry mton/hour	\$/mton	\$/dry mton
Grain (10% MC)	5.6	4.2	15.75	17.50
Cotton (20% MC)	3.3	2.6	110.20	137.75
Sugar cane (80% MC)	340	68	7.00	35.00

to improve the performance of their trucks and capture a greater share of this market.) Costs to operate the truck tractor are well defined.

It is interesting to compare the cost per dry t for the three systems. For this analysis, the cost to operate the truck is assumed to be \$450/day. Labor cost for the driver is included; however, fuel cost is not included.

If a truck averages three loads of 20% moisture content grain per day, and the load size is 22.5 t, the hauling cost is

$$3 \text{ loads/day} \times 22.5 \text{ t/load} \times (1 - 0.2) = 54 \text{ dry t/day}$$

$$\frac{\$450/\text{day}}{54 \text{ dry t/day}} = \$8.33/\text{dry t}$$

If a truck (module hauler) averages four loads of cotton per day and the module is 7.2 t at 20% moisture content, the hauling cost is

$$4 \text{ loads/day} \times 7.2 \text{ t/load} \times (1 - 0.2) = 23 \text{ dry t/day}$$

$$\frac{\$450/\text{day}}{23 \text{ dry t/day}} = \$19.56/\text{dry t}$$

The truck on which the module hauler is mounted is basically the same machine (same engine, drive train, running gear) as the truck tractor used for grain hauling; thus, it is appropriate to compare the hauling cost using the same per day truck cost.

If a truck averages 10 loads of sugarcane per day and the total load (two bins) is 24.5 t at 80% moisture content, the hauling cost is

$$10 \text{ loads/day} \times 24.5 \text{ t/load} \times (1 - 0.8) = 49 \text{ dry t/day}$$

$$\frac{\$450/\text{day}}{49 \text{ dry mton./day}} = \$9.18/\text{dry t}$$

These results are summarized in Table 7.9. Using the grain hauling as a reference, cotton is 2.3 times more expensive, and the sugarcane is 1.1 times more expensive. Unlike the harvest costs shown in Table 7.8, the hauling costs can be directly compared. *Truck ownership and operating cost (\$/day) for short-haul operations is approximately the same no matter what the truck is hauling.*

Table 7.9. Hauling cost for three commercial herbaceous biomass systems.

System	Cost	
	\$/mton	\$/dry mton
Grain (10% MC)	6.70	10.00
Cotton (20% MC)	19.55	15.65
Sugar cane (80% MC)	1.70	9.20

Receiving Facility

Grain is unloaded by positioning the hopper-bottom trailer over a unload pit and opening the hopper to allow the grain to flow into the pit. Unload time is about 5 minutes. The grain harvest season is so short that all farmers harvest the maximum amount each day to get their crop harvested before they are slowed by late fall weather delays. Often a queue of trucks forms waiting to be unloaded, thus it is typical for the unload time to average 30 minutes or more. There is a trade-off between the additional investment for a larger unload capacity and the farmer wait time. If the grain storage company invests more in the receiving facility, they have to charge the farmer more to store the grain to recover their cost.

The receiving facility typically does not delay operations at the cotton gin. Some gins haul in all modules they have under contract before they begin the ginning season. Most gins, however, begin ginning as soon as they have an inventory of modules, perhaps 100–500 modules depending on their gin capacity (number of modules ginned per day). The module haulers run continuously to keep the gin supplied. Sometimes they cannot keep up and the gin runs out of material. When this happens, the gin shuts down until the “at-gin” inventory is built back up.

The cotton gin is a mechanical process; it can be started and stopped with the throw of a switch. (This is a little bit of a simplification, but it can be stopped and restarted with less cost penalty than a sugar mill or bioenergy plant.) Gin owners want to gin the maximum number of bales per year, thus their goal is continuous operation.

Module haulers unload onto the ground in the storage yard, thus there is no waiting in a queue unless two trucks arrive at the scale at the same time. Even then, the delay is minimal. Operations at the gin receiving facility set the standard for all other biomass hauling operations. The key disadvantage of the gin system is that the truck only hauls one module, thus the load is only about 6 dry t.

The sugarcane receiving facility, because of the large number of trucks unloaded per day (typically about 1000 loads per day at one sugar mill in South Florida), is an excellent example of an optimized receiving facility operation. If the unloading station is clear (no truck unloading) when a truck arrives, it side-dumps the bins and immediately returns to the field. The typical time to weigh in, unload, and weigh out is about 3 minutes. If the truck proceeds to the storage area where the full bins are removed and empty bins are placed on the truck, this operation typically takes 3–4 additional minutes.

Design Constraints for a Bioenergy Plant Logistic System

How should the logistic system for a bioenergy plant be organized? It is appropriate to apply all that has been learned about the operation of existing biomass systems and develop a plan

for a logistic system that can supply herbaceous biomass to a bioenergy plant operating 24/7, 47 weeks per year. A woody biomass system, the fuel chip system, is a commercial reality and has already been described.

For the sample calculations we assume that the bioenergy plant will process 25 t/hour. Weekly demand is then

$$25 \text{ t/hours} \times 24 \text{ hours/day} \times 7 \text{ day/week} = 4200 \text{ t}$$

Annual demand is

$$4200 \text{ t/week} \times 47 \text{ week/year} = 197,400 \text{ t}$$

A truckload of fuel chips at 45% moisture content is about 12.4 dry t, thus it will require

$$\frac{4200 \text{ dry t/week}}{12.4 \text{ dry t/truck}} = 339 \text{ trucks/week}$$

If the receiving facility is open 5 days per week for 12 hours/day, the average operation will be 60 hours per week:

$$\frac{339 \text{ trucks/week}}{60 \text{ hours/week}} = 5 \text{ to } 6 \text{ trucks/hour}$$

Average unload time will need to be around 10 minutes.

A truckload of sugarcane at 80% moisture content is about 4.9 dry t, thus it will require

$$\frac{4200 \text{ dry t/week}}{4.9 \text{ dry t/truck}} = 857 \text{ trucks/week}$$

If the receiving facility is open seven days per week for 12 hours/day, the average operation will be

$$\frac{857 \text{ trucks/week}}{84 \text{ hours/week}} = 10 \text{ trucks/hour}$$

Average unload time will need to be about 6 minutes if the plant operates with only one unload station. Note that this comparison does not use the four-bin-load with two bins on the truck trailer and two on a second trailer.

Comparison of Harvesting Options

Two options have been proposed for the collection of herbaceous biomass as a feedstock for bioenergy, chopping (with a forage harvester), and baling (with a round baler or square baler). A logistic system for the forage chopping option has basically the same challenges as the sugarcane system. As previously mentioned, this system can work well for plantation agriculture but cannot be widely adapted in the United States. Several hundred farmers

chopping biomass and delivering on their own schedule to the bioenergy plant is not a practical option. If some on-farm storage option is used for the chopped material, perhaps a bunker silo, then the delivery can be better managed. Moisture content is an issue. The moisture must be high enough to achieve ensiling for acceptable storage, but hauling of silage means the hauling of large amounts of water, the same problem encountered with the sugarcane system.

Baling provides a disconnect between the harvest and infield operations, which is a significant advantage. One operator can bale an entire field with no requirement to coordinate with any other operation. If the big square baler is used, there is a requirement to haul the bales before they get rained on. (This requirement is not a major factor in the West, but it is a significant factor in the Southeast.) Once the big square bales get rained on and the water penetrates, it is unsafe to store the bales in covered storage. The big round bale can be stored in a single-layer ambient storage, because the rounded top thatches and sheds water. A hay shed with big square bales stacked about four high gives a storage cost of about \$8/t and a single-layer ambient storage of round bales on a gravel surface costs about \$2/t.

The round bale option is chosen for more detailed analysis. The key disadvantage of this option is that the round bale was developed to be used on the farm where it is produced. Systems for efficient over-the road hauling have not been developed.

The following constraints are listed for consideration by someone designing a round-bale logistic system.

1. The equipment should provide for multi-bale handling. The labor productivity of an operator on a machine loading individual bales, or unloading individual bales, is simply too low.
2. The equipment for over-the-road hauling must provide some increase in load bulk density over the bulk density of an individual bale. This increase may be modest, perhaps as low as 10%, depending on the cost to achieve it.
3. The goal for truck load time should be 10 minutes, meaning that a tractor-trailer truck is loaded with round bales in 10 minutes. If the cost (\$/t loading cost) to achieve this goal is too high in comparison with the truck cost reduction (\$/t) achieved with a 10 minute load time, then the optimum compromise between load cost and truck cost must be determined.
4. The goal for truck unload time should be 10 minutes, thus the interaction with the receiving facility at the bioenergy plant is critical. This goal equals the unload time for other materials (grain, fuel chips, sugarcane, and cotton).
5. The system must provide for easy flow of material into, and out of, at-plant storage. A system that gives the lowest delivered cost for feedstock through the plant gate may not give the lowest cost for a continuous stream of biomass into the plant 24/7.
6. Ideally, the equipment system must provide a means to establish a single-file stream of round bales into the plant. This requirement facilitates the introduction of the bales to the size reduction unit. Size reduction is best done at the plant.

Summary

Three herbaceous biomass logistic systems (cotton, sugarcane, grain) were compared with a woody biomass system (fuel chips). The linkage between harvesting, infield hauling,

and over-the-road hauling was explored. In general, productivity of an individual unit of equipment can be increased (less wait time) when the harvesting, infield hauling, and highway hauling operations are uncoupled. Sometimes this uncoupling is readily achieved, and sometimes it is overpowered by other factors in the overall system.

Two principles apply to all short-haul systems.

1. Truck productivity is maximized, and thus per t truck cost is minimized, when the load time and unload time is minimized. Load and unload time is defined to include time waiting in a queue.
2. Given the variability of field conditions, it is unlikely that just-in-time delivery of raw biomass will ever be practical, thus all logistic systems (herbaceous or woody) must provide for efficient flow of material into and out of at-plant storage. This is critically important for any plant that has a high-cost penalty for shutdown.

Systems Approach to Feedstock Logistics

It is common to design and analyze feedstock systems in discrete unit operations of harvest (mowing, raking, windrowing, and baling), storage, and transport, as done in certain sections of this chapter. However, looking at processes with a systems approach will give the best option. This involves examining the complete system to see what processes can be combined together for synergy of resources, reduction in waste, and cost reduction. For example, current technology looks at optimizing operations that harvest agricultural residues, or energy crops, and put the raw biomass into storage. The raw biomass is then put into the densest package practical for transport to a biorefinery for processing.

Another way to approach delivering biomass at least cost is to develop options that integrate systems together, thus achieving multiple tasks in one unit operation. For example, grain and fiber can be harvested together and brought to a farm-level location for subsequent separation of the grain and fiber. This system was discussed in the Biomass Harvest and Collection section. Another example is the development of a mobile pelleting machine that can densify feedstocks as they are harvested. Integrated systems provide new ways of viewing the problem and formulating the correct design questions.

Integrated systems are increasingly becoming the way to design systems and analyze pathways. In agriculture and life sciences, this is made possible by the advances made in genetic engineering. Biomass plants engineered with enzymes in their tissue can undergo pretreatment while in storage, thus eliminating harsh pretreatment and saving energy at the plant. These innovative ideas are often not easy to put together in reality. A bottleneck to developing integrated systems is the lack of close collaboration between all expertise in the production chain. With each discipline focused on their own aspect of the chain, a lot of opportunities for synergies are lost. Collaboration allows everyone to see the bigger picture of how everything fits in place and therefore harness the strengths of the production chain as a whole. An integrated systems approach can lead to optimal design of systems and processes. Because of the complexity and diverse disciplines involved in the production and use of agricultural feedstocks and waste for energy, it is even more important to approach problem formulation within an integrated systems approach.

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Chapter 8

Conversion of Existing Dry-Mill Ethanol Operations to Biorefineries

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Abstract

Basic corn-to-ethanol manufacturing processes have provided important first steps for bio-refining operations but have barely scratched the surface with respect to overall biorefining opportunities. Multiple options exist to modify or supplement existing processes to make these plants more productive and increase the types and quantities of valuable materials that they produce. Some low-value byproducts and wastes generated from these facilities can be converted into higher value products. Additionally, byproducts and wastes from other industries, such as food processing, landscaping, paper, and municipal solid waste facilities, could be substituted for crops as feedstocks and processed into ethanol. This chapter focuses on two incremental modifications that dry-mill ethanol plants could implement that would enlarge their feedstock options and also expand the products and associated value of their output. The proposed modifications include (1) incorporation of cellulosic feedstocks into existing operations and (2) recovery of oil for sale as a value-added product. Modification of existing processes to accommodate cellulosic feedstocks could greatly improve the diversity and flexibility of feedstock options. Recovery of oil from by products such as germ, thin stillage syrup, or dried distillers' grains and solubles (DDGS) could expand greatly the quantities and value of products produced from dry-mill plants and also provide valuable feedstock options for biodiesel producers. Multiple other opportunities exist for expanding and diversifying dry-mill ethanol plant feedstocks, processes, and products but are beyond the scope of this chapter. For instance, DDGS could be further fractionated to separate and pelletize high-protein/high-value components from lower value materials. Cogeneration systems could be implemented to burn lignin and other coproducts to simultaneously produce steam and electricity, thereby reducing electricity requirements from external sources and providing electrical power for additional biorefining operations. Ethanol is an important industrial ingredient and has widespread use as a base chemical for other organic compounds. These include ethyl halides, ethyl esters, diethyl ether, acetic acid, butadiene, and ethyl amines.

Introduction

Today's ethanol industry is frequently criticized for being resource intensive in terms of energy, water, grain, fertilizer, and other inputs required for production. While the ethanol produced at these facilities is a very valuable fuel, numerous opportunities exist to reduce waste and expand the diversity of both the inputs and the outputs associated with their operations. Multiple options exist to modify or supplement existing processes to make these plants more productive and increase the types and quantities of valuable materials that they produce. Some low-value byproducts and wastes generated from these facilities can be converted into higher value products. Additionally, byproducts and wastes from other industries, such as food processing, landscaping, paper, and municipal solid waste facilities, could be substituted for crops as feedstocks and processed into ethanol. This would reduce the strain on food resources commonly associated with biofuels production.

Basic corn-to-ethanol manufacturing processes have provided important first steps for biorefining operations but have barely scratched the surface with respect to overall biorefining opportunities. This chapter focuses on two incremental modifications that dry-mill ethanol plants could implement that would enlarge their feedstock options and also expand the products and associated value of their outputs. The proposed modifications include (1) incorporation of cellulosic feedstocks into existing operations and (2) recovery of oil for sale as a value-added product.

The United States currently converts approximately 15 million tons of agricultural products into ethanol and biodiesel and discards approximately 270 million tons of agriculturally derived residues in the form of harvestable crop residues, animal manure forest residues, and the organic fraction of municipal solid wastes (Baker 2006). As of February 2006, the annual capacity of the U.S. ethanol sector stood at 4.4 billion gallons, and plants under construction or expansion are likely to add another 2.1 billion gallons to this number (Clements and Van Dyne 2006). According to the U.S. Department of Agriculture Agricultural Baseline Projections (released in February 2006), the share of ethanol in total corn use will rise from 12% in 2004–2005 to 23% in 2014–2015. A comparison of the 2006 Baseline with the 2005 Baseline suggests that much of the increased use by ethanol producers will be diverted from potential exports because the 2006 Baseline projects higher use for ethanol and lower exports than the 2005 Baseline.

With a corn-to-ethanol conversion rate of 2.7 gallons per bushel (a rate that many state-of-the-art facilities are already surpassing), the U.S. ethanol sector will need 2.6 billion bushels per year by 2010, which is 1.2 billion bushels more than it consumed in 2005 (U.S. Department of Agriculture, Economic Research Service 2006) Adaptation of the market to this increased demand is likely to be one of the major developments of the early 21st century in U.S. agriculture.

Current Processes

Most ethanol is currently produced from corn using a dry-milling process. Conventional dry-mill ethanol production is a relatively simple procedure. Corn is cleaned, tempered with steam, ground, and wetted to a free flowing "mash." The mash is superheated in a cooker where acid and/or enzymes are added to solubilize starch. Water is added to adjust solids concentration and temperature along with saccharifying enzymes. The mash is placed in a fermenter, and yeast is added to convert sugars to alcohol. When the fermentation is complete,

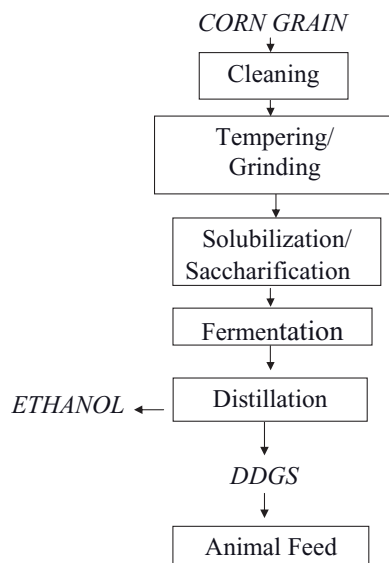


Figure 8.1. Dry-mill process.

alcohol is removed by distillation and the residual “still bottoms” are recovered for animal feed. In such a plant, the only two products are ethanol and DDGS. Some plants now separate the germ before “mashing.” Food-grade corn oil can then be extracted from the germ, thus creating another salable product. Figure 8.1 provides a basic process flow diagram that describes the dry-milling process.

The rising demand and cost of corn has challenged the economic feasibility of conventional dry-mill processing in recent years. Conversion and retrofitting existing dry-mill ethanol plants such that they can process cellulosic biomass materials is becoming a topic of considerable interest. Most ethanol plants have been located in relatively rural settings. Therefore, access to relatively low-cost land is frequently available to allow for expansion of processing equipment as well as additional options for storing feedstocks and products. The DDGS produced in the dry-mill process has considerable value as livestock feed. However, practical methods for improving the value of this material and extracting additional marketable products from the DDGS could greatly improve the economic viability of dry-mill ethanol plants.

This chapter discusses relatively nondisruptive methods for incorporating cellulosic feedstocks into existing dry-mill ethanol plants using existing off-the-shelf technology. Additionally, practical techniques for extracting marketable oil suitable for food, feed, or conversion to biodiesel will be discussed. By expanding both the feedstock options and the marketable products that can be produced at these facilities, dry-mill ethanol plants can improve their long-term competitiveness and viability.

Cellulosic Feedstocks

Cellulosic materials have been touted as the feedstock for the “second generation” of biofuel production and the best alternative for replacing food and feed grains in ethanol production.

Proponents of cellulosic ethanol point out that its production generates a higher net energy gain and a lower level of greenhouse gas emissions relative to grain-based ethanol, due in part to the fact that a higher portion of the feedstock material is converted to fuel. As a result, the past decade has seen a tremendous increase in research related to ethanol production from feedstocks such as corn stover, switchgrass, rice hulls, wheat straw, landscape waste, paper processing waste, wood-processing waste, and sugarcane waste (U.S. Department of Energy, National Renewable Energy Laboratory 2007).

The U.S. Department of Energy committed more than \$1 billion toward cellulosic ethanol projects in 2007, with a goal of making the fuel cost competitive at \$1.33 per gallon by 2012 (U.S. DOE 2007). Projects supported by the 2007 DOE commitment range from annual capacities of 11 million gallons of ethanol (Kansas) to 125 million gallons (Iowa) (U.S. DOE 2007). The technologies utilized by these proposed plants also vary, as do their feedstocks. Feedstocks expected to be used by some of these proposed ventures include corn stover and cobs, rice and wheat straw, milo stubble, switchgrass, yard waste, wood and wood-processing residues, "green" wastes, and other wastes recovered from landfills. Some of the technologies to be utilized also generate coproducts such as electricity, hydrogen, ammonia, and methanol (U.S. DOE 2007).

A wide variety of cellulose-based biomass wastes and byproducts are available for conversion to biofuels. These include:

- Agricultural residues (corn stalks and cobs, straws, cotton gin trash, palm oil wastes, etc.)
- Paper (paper mill sludge, recycled newspaper, sorted municipal solid waste, etc.)
- Wood waste (sawdust, wood chips, prunings, etc.)
- Landscape waste (leaves, grass clippings, vegetable and fruit wastes, etc.)

Most of these materials are available at very low cost, and some even command tipping fees associated with their disposal as wastes.

Unlike grain-based ethanol, where processing technologies have become relatively standardized and feedstock procurement is as simple as participating in the grain marketing system, cellulosic ethanol projects may have a wide range of technical efficiencies, conversion rates, and feedstock logistics. Decision makers, including agricultural producers, potential investors, and rural community leaders, are interested in determining whether cellulosic ethanol production could be feasible in their area.

Cellulosic biomass is composed of cellulose, hemicellulose, and lignin. In order to produce ethanol from cellulosic biomass, complex cellulosic carbohydrates must be converted into simple sugars, which can then be fermented to ethanol by a variety of microorganisms. Cellulose conversion to sugars can be catalyzed by a variety of acids, including sulfuric, hydrochloric, hydrofluoric, and nitric acids. A decrystallized cellulosic mixture of acid and sugars reacts in the presence of water to produce individual sugar molecules (hydrolysis). The product from this hydrolysis is then neutralized, and yeast fermentation is used to produce ethanol. When inexpensive dilute acid is used to catalyze the hydrolysis reaction, biomass is impregnated with dilute sulfuric acid solution and treated with steam at temperatures ranging from 140 to 260°C (Katzen and Schell 2006). Concentrated acids can also be used to hydrolyze cellulose and hemicellulose to sugars.

Temperatures ranging from 100 to 120°C, which are lower than those with the dilute acid process, are typically used, and high yields of sugars are obtained with little production of degradation products. The economic viability of this process depends, however, on the successful recovery of the acid at low cost (Katzen and Schell 2006).

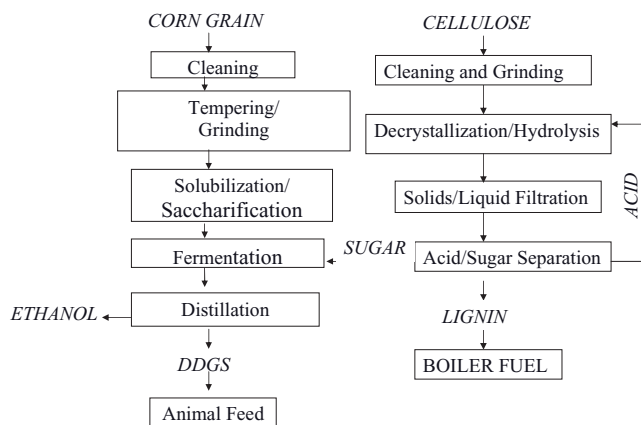


Figure 8.2. Dry-mill process combined with cellulose hydrolysis processing.

Enzymatic conversion of cellulose to sugars offers some promising advantages over acid hydrolysis. Sugar yields are limited during acid hydrolysis because sugars are also converted to degradation products. Cellulase is a multicomponent enzyme system that catalyzes cellulose hydrolysis and is 100% selective for conversion of cellulose to glucose; high yields are therefore possible. This enzyme is produced by a variety of microorganisms, most commonly, the fungus *Trichoderma reesei*. Cellulose conversion rates are limited by the ability of the enzyme to access the cellulosic substrate. To increase accessibility, biomass is subjected to physical and chemical treatments that disrupt the biomass structure, usually by removing a fraction of the hemicellulose and/or lignin. Effective pretreatment is necessary to achieving good cellulose-to-glucose conversion yields (Katzen and Schell 2006).

Figure 8.2 provides a process flow that describes how cellulose processing might be incorporated into an existing dry-mill ethanol production facility. With this configuration, the plant could continue to process corn feedstocks but could also have the capability of processing cellulosic feedstocks. Cellulosic feedstocks would be cleaned and ground to reduce particle size and expedite processing. The material would then be dried as needed to a moisture content acceptable for acid decrystallization (separation of the cellulose and hemicellulose from the lignin).

In Figure 8.2 example, a process using concentrated sulfuric acid is described. The concentrated acid process offers some advantages over the dilute acid process. Even though the dilute acid is considerably cheaper to purchase, the concentrated acid process can compete successfully if the acid is recycled. While this would add capital expenditure up front, it would significantly reduce operating costs through lower expenditures for acid. Additionally, the concentrated acid process operates at lower temperatures (100–120°C) than the dilute processes, which operate at 140–260°C (Katzen and Schell 2006). Therefore, considerably less energy is required for the concentrated acid process.

In the near term, the concentrated acid process offers higher production rates than enzymatic processes. However, enzymatic processes are safer and more environmentally friendly than those that use concentrated acid. Consequently, as the productivity of enzymatic hydrolysis processes are improved, it is likely that the long-term prospects for this technology will be more favorable than either dilute or concentrated acid hydrolysis processes.

Conventional corn-to-ethanol conversion processes, baker's yeast (*Saccharomyces cerevisiae*), is commonly used in the fermentation step to produce ethanol from hexose (six-carbon sugar). The carbohydrates present in lignocellulosic biomass are considerably more complex than those derived from corn. Large quantities of xylose and arabinose, which are five-carbon sugars derived from the hemicellulose portion of the lignocellulose, are also present in the products derived from hydrolysis. For instance, when corn stover is hydrolyzed, approximately 30% of the total fermentable sugars produced are in the form of xylose. Consequently, the fermenting microorganisms used in cellulosic ethanol production must be capable of utilizing the entire range of sugars produced during hydrolysis. This will be vital to increasing the economic competitiveness of cellulosic ethanol and to incorporating cellulosic ethanol processes into existing corn-to-ethanol operations.

Metabolic engineering for microorganisms used in fuel ethanol production has made significant progress in recent years. In addition to *S. cerevisiae*, microorganisms such as *Zymomonas mobilis* and *Escherichia coli* have been targeted through metabolic engineering for cellulosic ethanol production (Jeffries and Jin 2004). Engineered yeasts have also been shown to effectively ferment xylose (Ohgren et al. 2006) and arabinose (Becker 2003) as well as both sugars together (Karhum et al. 2006). Utilizing yeast cells for cellulosic ethanol processes is particularly appealing because they have been used in biotechnology for hundreds of years. Yeast is tolerant to high ethanol and inhibitor concentrations because they can grow at low pH values, which prevent bacterial contaminations.

In order to successfully incorporate cellulosic ethanol production using concentrated acid hydrolysis into existing dry-mill processing, several key factors must be addressed. Concentrated acid is expensive; therefore, efficient methods for recovering and reconcentrating acid must be incorporated. The sugars produced through the hydrolysis process must be of high concentration and high purity. Additionally, the process must have the ability to ferment both six-carbon and five-carbon sugars efficiently with conventional microbes. It is anticipated that early attempts to incorporate acid hydrolysis of cellulosic materials into existing dry-mill operations will include dedicated fermentation vessels that are separate from the fermenters that process corn-derived sugars. As yeast strains that are equally capable of fermenting both six-carbon and five-carbon sugars are developed, the need for dedicated fermenters will no longer be necessary.

Several technologies that have been proven in small-scale facilities have been developed to produce cellulosic ethanol. These technologies are moving toward commercial production. However, challenges remain with respect to expanding the technologies to production scale, reducing production costs, and financing large-volume plants. To spur commercial development, the U.S. DOE announced grants of \$385 million for six commercial-scale cellulosic ethanol biorefineries in February 2007. Chesterfield, Missouri-based Abengoa Bioenergy, and Range Fuels in Broomfield, Colorado, were each awarded \$76 million; BlueFire Ethanol in Irvine, California, received \$40 million; and Sioux Falls, South Dakota-based Poet was granted \$80 million. These companies expect to complete commercial-scale facilities between 2009 and 2011. The two remaining firms, Alico, in La Belle, Florida, and Iogen in Ontario, Canada, which were awarded \$33 million and \$80 million respectively, have dropped out of the program (Greer 2008). The grants were awarded under Section 932 of the Energy Policy Act of 2005, which authorized the DOE to fund commercial demonstration of advanced biorefineries that use cellulosic feedstock to coproduce ethanol, bioproducts, heat, and power. Awards were capped at 40% of the total project cost, up to a maximum of \$80 million (Greer 2008).

BlueFire Ethanol is developing a \$150 million cellulosic ethanol facility in Riverside County, California. The so-called Mecca project will use concentrated acid hydrolysis to convert the green waste portion of a municipal solid waste stream and green agricultural waste, currently sent to landfills, into 17 million gallons per year of ethanol. The firm expects to begin construction in late 2009.

The first step in BlueFire's process, which is described on their Web site, employs concentrated acid hydrolysis to separate the cellulose and hemicellulose from the lignin and then hydrolyze the cellulose to produce simple sugars for fermentation (BlueFire Ethanol, n.d.). After hydrolysis, a filtration and pressing process will remove the lignin and other insoluble materials from the sugar mixture.

BlueFire's process utilizes a chromatographic system to separate the acid from the sugar. The process concentrates and recycles 98% of the sulfuric acid for reuse. The remaining 1%–2% of the acid left in the sugar solution is neutralized with lime, creating hydrated gypsum that can be easily separated from the sugar solution. Specially developed cultures of yeasts are then used to ferment the sugar stream into ethanol.

Byproducts from the process include lignin, which will be burned in solid fuel boilers to satisfy about 70% of the plant's thermal needs, a yeast stream that can be sold into animal feed markets, and agricultural-grade gypsum that can be used as a soil amendment. The project's proximity to Los Angeles is advantageous in reducing the cost of transporting feedstock to the plant and moving the ethanol to market.

Constructing this type of plant in California offers advantages over most other locations because curbside source separation and primary separation of municipal solid waste materials has already been implemented to a great extent. Feedstock used in the process includes green agricultural waste, commercial landscaping green waste, clean woody construction and demolition debris, and short paper fibers that cannot be recycled.

Poet and Abengoa Bioenergy plan to utilize enzymatic hydrolysis and fermentation techniques to produce cellulosic ethanol. The processes employ enzymes to liberate fermentable sugars locked in the complex carbohydrate structures that form the cell walls of plants. Microbes then ferment the sugars into ethanol (Greer 2008). Poet will convert an existing 50 million gallons per year corn ethanol facility in Emmetsburg, Iowa, into a 125 million gallons per year biorefinery, which will include a 25 million gallons per year cellulosic ethanol processing system. The new \$200 million facility, called Project Liberty, will produce cellulosic ethanol from 770 tons of corncobs and corn fiber. Construction is expected to start in early 2010, with completion and commissioning in the second half of 2011 (Greer 2008).

Utilizing corncobs as a source of feedstock offers a less disruptive source of biomass than most other byproducts, wastes, and crops. In conventional harvesting systems, corn is harvested by combines and the kernels are removed from the cob. The cobs are discarded and left on the surface, where they are plowed into the soil at a later date. By collecting the cobs separately from the kernels during harvesting, a relatively uniform source of biomass can be removed and stored for conversion to ethanol. The remainder of the crop residues can be left for incorporation into the soil and preservation of organic matter levels.

Poet estimates that farmers will receive between \$30 and \$60 per ton of corncobs and the average acre of corn yields between three-quarters to a ton of corncobs. Each ton of corncobs will produce approximately 85 more gallons of ethanol per acre. Consequently, about 27% more ethanol can be produced by adding the cellulosic production method to the current corn-to-ethanol technology (Greer 2008).

Colocating the corn and cellulosic ethanol facilities will allow Poet to leverage its relationships with the hundreds of farmers that already provide corn to the plant. Those same farmers

will provide the cobs as well. The cellulosic ethanol facility will also take advantage of the existing biorefinery infrastructure, including roads, railroads, utilities, and land.

Waste from the cellulose-to-ethanol process will be used to produce steam in a solid fuel boiler and biogas in an anaerobic digester, generating process heat for the entire biorefinery. These alternative fuels will significantly reduce Poet's usage of natural gas. The company completed a \$9 million pilot-scale cellulosic ethanol plant, adjacent to its 9 million gallons per year corn ethanol refinery in Scotland, South Dakota, in late 2008. The facility will produce 20,000 gallons of cellulosic ethanol from corncobs and fiber. Lessons learned from the development, testing, and validating of the technology at the Scotland facility will be applied to the design and engineering of the project (Greer 2008).

Abengoa Bioenergy is also building a hybrid biorefinery producing corn and cellulosic ethanol. The new facility in Hugoton, Kansas, will produce 85 million gallons per year of corn ethanol and 11.4 million gallons per year of cellulosic ethanol from 400 dry metric tons of biomass. Total project costs are estimated at \$500 million, including \$190 million for the cellulosic ethanol plant. Abengoa currently produces 198 million gallons per year of corn ethanol in the United States and 142 million gallons per year in Europe. The company also operates a cellulosic ethanol pilot facility in York, Nebraska.

Initially, biomass feedstock for the Hugoton plant will include corn stover, wheat straw, and milo stubble. In an effort to develop feedstock sources that are diverse and sustainable, the project will work with local farmers to establish energy crops, such as switchgrass, on nonfood-producing acres. A biomass gasification system producing syngas for thermal energy will reduce fossil fuel usage and greenhouse gas emissions.

Abengoa has received \$15 million of its \$76 million DOE grant to fund the preliminary design, permitting, and environmental review. Construction will begin shortly thereafter, with completion slated for 2011.

Oil Recovery

Recovery of oil from ethanol processing byproducts offers some very promising opportunities for dry-mill plants to expand products and markets. DDGS has become a very valuable live-stock feed in recent years but possesses some characteristics that limit its use in some applications. DDGS contains about 10.5% oil, which is about three times the level found in most corn. This relatively high oil content limits its use as a feed supplement particularly with respect to swine (Plain 2006). Additionally, the high oil content reduces DDGS longevity with respect to storage and shipping because the oil can become rancid, rendering the DDGS unsuitable for use as feed.

A 56-lb bushel of corn produces 2.72 gallons of ethanol and approximately 17 lb of distillers' grains in various forms (Renewable Fuels Association 2008). It is estimated that approximately 2.6 billion bushels of corn will be used to produce ethanol per year by 2010 (Baker and Zahniser 2006). About 2/3 of that corn will be used in dry-milling operations (Murthy et al. 2004). Consequently, an anticipated 29 billion pounds of DDGS will be produced from dry-mill plants. The DDGS contains about 10.5% oil, and the oil weighs about 7.6 lb per gallon, so about 400 million gallons of oil is potentially available for alternative uses if it could be separated from the DDGS (about 0.085 gallons of oil for every gallon of ethanol produced). For comparison purposes, a single dry-mill ethanol plant that produces 100 million gallons of ethanol annually would have about 8.5 million gallons of oil available for separation and/or extraction.

The oil can be removed from fractionated germ prior to fermentation, in which case it is suitable for human consumption. Alternatively, the oil can be removed from DDGS after fermentation. In this case, the oil would be unfit for human consumption because of its relatively high levels of free fatty acids (about 8%–9%). However, the oil could be used as animal feed or as feedstock in the production of biodiesel (methyl ester). Given that biodiesel production was 495 million gallons in 2007, the oil contained in DDGS offers considerable potential as a feedstock for biodiesel production without committing additional acreage to biofuel crops (ICM 2009).

Corn kernels are composed of four primary parts. The endosperm is the largest component (about 82%) of the kernel and is made up primarily of starch and protein. This starch fraction is the portion that is fermented into ethanol. The germ is the next largest fraction at 12% of the kernel and is the primary source of corn oil. The pericarp is the seed hull and composed 5% of the kernel, while the tip cap is where the seed was attached to the cob and makes up about 1% of the kernel. Wet-milling processes can efficiently fractionate the kernels and remove the oil-containing germ from the fermentable fraction prior to producing ethanol, thus allowing for the extraction of food-grade oil from the germ. Dry-mill plants can also be retrofitted to remove the germ prior to fermentation. This is accomplished by either (1) quick germ (and quick germ quick fiber) methods or (2) enzymatic milling. In the quick germ process, the whole corn is soaked in water for 3–12 hours at 60°C. Soaking the ground corn in water with enzymes increases specific gravity such that the germ and fiber float prior to fermentation. After soaking, the corn, a conventional Bauer mill, is used for degermination, similar to the methods used in the wet-milling process. The germ is recovered using germ hydroclones, and the rest of the corn is ground and processed through the dry-mill process (Singh and Eckhoff 1997).

In enzymatic milling, soaking is followed by incubating with protease and starch-degrading enzymes for 2–4 hours. After incubation, quick germ processes are used to recover germ and pericarp fibers. The remaining slurry is screened on a 200-mesh sieve to recover endosperm fibers. In either case, separation of the germ allows for the recovery of high-value food-grade corn oil from the germ (Singh et al. 2005). This can be accomplished through the use of an expeller and/or solvent extraction. Removal of endosperm fibers will further increase fermentation capacity and reduce fibers in the DDGS and increase the protein content of the DDGS, making the DDGS more suitable for a wider variety of livestock applications. Also, these modified milling processes can produce additional ethanol per batch because non-fermentables (germ, pericarp fibers, and endosperm fibers) are removed. These non-fermentables can be replaced by a more fermentable substrate. Plants performing these modified milling processes can potentially increase the amount of corn processed and therefore produce more ethanol per batch compared with conventional dry-mill process (Singh et al. 2005).

Retrofitting an existing dry-mill plant to remove the germ prior to fermentation using either the quick germ or enzymatic milling processes is a relatively involved and costly endeavor requiring significant modifications and capital investment in equipment. However, the improvements associated with ethanol production, along with the high value of coproducts such as the food-grade corn oil produced and the improved quality of the DDGS, can justify the investment. Consequently, ethanol production technology providers such as Mercer Energy, FWS, FCStone Carbon, and ICM now offer fractionation technology to their clients with dry-mill plants.

Two primary options, centrifugation and solvent extraction, are available to dry-mill ethanol producers that can facilitate recovery of oil from either individual process streams or the combined DDGS byproduct. The centrifugation method is relatively straightforward,

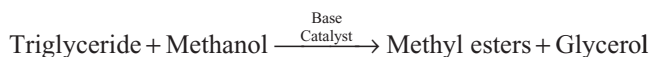
requiring a relatively low capital investment (\$500,000 to \$1 million to implement for an average ethanol plant). Solvent extraction methods are considerably more involved, require considerable capital investment, and create hazards that require careful consideration prior to implementation.

During the distillation process, solids comprising the grain and added yeast, as well as liquid from the water added during the process, accumulate in the bottom of the distillation tanks (ICM 2009). The solids are processed through centrifuges for separation into thin stillage (a liquid with 5%–10% solids) and wet distillers' grain. Some of the thin stillage is routed back upstream in the process for use as makeup water, reducing the amount of fresh water required. The rest is sent through a multiple-effect evaporation system where it is concentrated into syrup containing 25%–50% solids. This syrup, which is also high in protein and oil content, is then mixed back in with the distillers' grain and further processed to create animal feed. In an effort to recover the valuable oil, some facilities have added a centrifugation step to the syrup prior to mixing it with distillers' grain (ICM 2009).

The thin stillage syrup contains about 3.5% to 7% oil, depending on moisture content. Centrifugation will remove about 1/3 of the oil contained in the syrup. Therefore, a 60 gallon per minute centrifuge (the size implemented by ethanol plants that produce 50 to 100 million gallons of ethanol per year) could realistically produce about 500,000 to 1 million gallons of oil per year. Implementation of centrifugation to remove oil from the syrup is a relatively inexpensive and nondisruptive process change. However, centrifugation of the thin stillage syrup would only recover about 10% to 15% of the total oil available from the entire plant. The remaining oil is present in the DDGS and requires extraction with a solvent-based process.

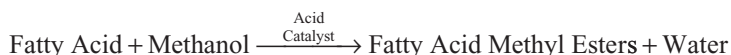
Solvent extraction systems are much more efficient methods with respect to removing nearly all of the available oil from either the germ or DDGS. However, they tend to be complex and hazardous to operate because of the flammable solvents used for extraction. With solvent extraction processes, the germ or DDGS is fed into an extractor, where the material forms a uniform shallow bed and is washed with a solvent such as hexane as it is conveyed across the upper, horizontal section of the extractor, counter current to the solvent. The concentrated oil–solvent mixture discharges from the extractor through a hydroclone. The hydroclone “scrubs” the fines from the oil–solvent mix before being pumped further to a distillation system. On a typical extractor, there are seven stages of oil–solvent mixture, ranging from about 2% oil concentration to approximately 25%. From the extractor, the concentrated oil–solvent mixture is sent to a distillation system, where the oil and solvent are separated. The solvent is recycled back into the extraction process and the oil is available for sale or for conversion into other products. De-oiled DDGS would be available for markets that need less oil for the livestock it will be fed to, or for applications that require long-term storage or shipping.

Most conventional large-scale biodiesel plants use base-catalyzed transesterification to convert triglyceride in the form of vegetable oil to methyl ester (Steinbach 2007).



This process alone is not suitable for processing oil derived from DDGS because of the relatively high levels of free fatty acids present in the oil. The base catalyst converts the free fatty acids to soap, thus reducing the yield of methyl ester. Consequently, the base-catalyzed process must be supplemented with a pretreatment step with an acid catalyst to convert the

fatty acids to methyl ester. Following the pretreatment step, the remaining triglycerides can be converted to methyl ester using the base-catalyzed process.



Biodiesel is now well accepted as a petroleum diesel fuel alternative offering multiple advantages such as renewability, energy security, and superior environmental performance. It is anticipated that feedstocks for biodiesel production will be increasingly in short supply in coming years. The number of biodiesel producers increased by more than 400% from 2004 to 2007. The total biodiesel production capacity available in the United States, as of January 2008, was 2.2 billion gallons from 171 plants, of which 137 came on line in 2004 (Steinbach 2007). Less than 1/2 billion gallons (less than 25% capacity) was actually produced in 2007 partly due to issues associated with feedstock availability (particularly soybean oil) and price. Developing alternative sources of feedstocks will become increasingly important as these plants attempt to increase their production to levels near their capacity.

Summary

Basic corn-to-ethanol manufacturing processes have provided important first steps for biorefining operations but have barely scratched the surface with respect to overall biorefining opportunities. Numerous opportunities exist to reduce waste and expand the diversity of both the feedstocks and the products associated with these operations. Byproducts and wastes from various industries, such as food processors, landscapers, paper processors, and municipal solid waste facilities, could be substituted for crops as feedstocks. These materials can be processed into ethanol, thus reducing the strain on food resources commonly associated with biofuels production. Additionally, some low-value byproducts and wastes generated from these facilities can be converted into higher value products. This chapter describes two incremental opportunities for implementing existing technology that would enable expansion of existing dry-mill ethanol operations with respect to feedstocks and products. Modification of existing processes to accommodate cellulosic feedstocks could greatly improve the diversity and flexibility of feedstock options. Recovery of oil from byproducts such as germ, thin stillage syrup, or DDGS could expand greatly the quantities and value of products produced from dry-mill plants and also provide valuable feedstock options for biodiesel producers.

Multiple other opportunities exist for expanding and diversifying dry-mill ethanol plant feedstocks, processes, and products but are beyond the scope of this chapter. For instance, the DDGS could be further fractionated to separate and pelletize high-protein/high-value components from lower value materials. Cogeneration systems could be implemented to burn lignin and other coproducts to simultaneously produce steam and electricity, thereby reducing electricity requirements from external sources and providing electrical power for additional biorefining operations. Ethanol is an important industrial ingredient and has widespread use as a base chemical for other organic compounds. These include ethyl halides, ethyl esters, diethyl ether, acetic acid, butadiene, and ethyl amines (Dipardo 2008). Adding additional processes to convert ethanol to these other value-added products could provide solid opportunities for expanding operations in the future.

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Chapter 9

Cellulosic Ethanol from Agricultural Residues

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Abstract

Cellulosic biomass is inexpensive and abundant and provides a unique resource for large-scale and low-cost solar energy collection and storage. Agricultural residues are particularly promising for initial commercial applications because of their potential low cost and near-term availability. Because the rapidly evolving tools of biotechnology can radically lower conversion costs and enhance yields, biological processing presents a particularly promising approach to converting these solids into liquid fuels that better fit our transportation infrastructure while providing unparalleled environmental, economic, and strategic benefits. Yet breakdown of the cellulose and hemicellulose in these naturally resistant cellulosic materials to release fermentable sugars is projected to be the most expensive processing step. In addition, the pretreatment step needed to realize high yields has pervasive impacts on all other major operations from choice of feedstock through product recovery and residue processing. Thus, knowledge of how agricultural residues respond to pretreatment and integrate with other operations is vital to successful applications. This chapter begins with an overview of biological processing of agricultural residues to ethanol followed by a summary of environmental considerations in their use and some estimates of availability based on these factors. Information is also given on the composition of major agricultural residues and reported yields of sugars from many such materials to provide a perspective on their suitability for ethanol production. Then, approaches and needs for harvesting, transporting, and storing agricultural residues are discussed. The chapter closes with a simplified analysis of the cost of processing cellulosic biomass to ethanol to point out key cost factors and the importance of employing low-cost feedstocks and realizing high yields. In addition, opportunities for advanced technologies to lower the cost of biological processing to ethanol and other products are outlined.

Introduction

Cellulosic biomass provides a truly unique resource for large-scale sustainable production of liquid fuels that integrate into our existing transportation infrastructure, and no other raw material can match its potential impact and cost (Lynd et al. 2008). For example, cellulosic biomass costing about \$63/dry ton is as inexpensive as petroleum at \$20/barrel on an equivalent energy content basis (Lynd et al. 1999). Furthermore, the U.S. Departments of Agriculture and Energy estimate that over 1.3 billion dry tons of biomass could be available annually, of which about 1 billion dry tons per year are agricultural resources available on the sustainable basis (Perlack et al. 2005; Long 2008). This quantity is enough to make a major impact on energy supplies (Lynd et al. 2008), with the result that conversion of agricultural biomass to organic liquid fuels (e.g., ethanol) can enhance energy security, reduce trade deficits, enhance global competitiveness, and create rural employment. In addition, processing cellulosic biomass to ethanol can continue to employ the power of biotechnology to simplify technology and realize high yields vital to low costs that address concerns about mounting petroleum prices (Wyman 1993, 1994b, 1999a). Because transportation is the single largest contributor to carbon dioxide (CO₂) emissions in the United States (Tyson 1993; Wyman 1994a; Farrell et al. 2006; U.S. DOE 2006), the promise for cellulosic fuels to reduce greenhouse gas (GHG) emissions by about 90% and more compared to gasoline coupled with the low-cost potential and large resource base are vital as we seek avenues to abate increasing temperatures and deterioration in the climate. In fact, the only other potentially cost-effective energy options to power mobility with a low carbon footprint are through energy storage in batteries, hydrogen, or compressed air, provided the electricity required to power each is derived from sustainable technologies at low cost. Even then, liquid fuels will be essential for long distance transport and aircraft. Combining cellulosic fuels with plug-in hybrids, more public transportation, and better fuel efficiency will likely prove the most cost-effective avenue to affordable local and long distance mobility with low carbon emissions.

Despite its great promise and tremendous progress in improving cellulosic conversion technology, no commercial facilities are yet in place, with a vital challenge being to overcome the perceived risk of implementing the technology for the first time (Wyman 1999a). Once commercialized, costs are expected to drop dramatically through the learning curve effect, as clearly demonstrated for cane sugar ethanol in Brazil and corn ethanol in the United States, and projects will become both more profitable and less risky as more capacity comes on line (Wyman 2007). The current situation presents a classical chicken-and-egg challenge of how to overcome the greater risk and lower returns associated with first commercial plants to realize lower costs and higher returns of mature projects.

Because feedstock costs are dominant in processing economics, it is critical to seek those that are low in cost for first applications while being sufficiently abundant. However, high product yields and ease of processing are also vital to minimizing costs, while sufficient amounts must be available to support a large enough facility to achieve reasonable economies of scale. Agricultural residues are expected to serve as a major biofuels feedstock, and their potential low cost and current availability can be particularly important in the near term (Perlack et al. 2005). Thus, this chapter will summarize estimated amounts of leading agricultural residues and their potential for making ethanol. However, first an overview will be presented of the biological conversion of these materials to ethanol to provide a context on key feedstock and processing considerations. The economics of converting residues to ethanol will then be outlined to demonstrate the importance of feedstock composition, availability, and cost to good returns on capital. In addition, some other important

considerations in process economics and financing will be summarized. Finally, strategies will be discussed to introduce technologies for biological conversion of agricultural residues to ethanol.

Biological Processing of Cellulosic Biomass to Ethanol

Biological conversion of cellulosic biomass to ethanol is summarized here to provide a context for understanding some of the key features of agricultural residues and their impact on producing fuels, with more details available elsewhere (Valkanas et al. 1977; Fan et al. 1981; Gould 1984; Gusakov et al. 1992; Saddler 1993; Asghari et al. 1996; Baghaei-Yazdi et al. 1996; Belkacemi et al. 1997; Walsh et al. 1998). Typically, cellulosic biomass is composed of about 40%–50% cellulose, 20%–30% hemicellulose, 10%–25% lignin, and lesser amounts of minerals, oils, free sugars, starches, and other compounds (Wyman 1996). Enzymes or acids can catalyze the reaction of hemicellulose with water to release sugars, typically arabinose, galactose, glucose, mannose, and xylose, for fermentation to ethanol or other products. Enzymes or acids can also hydrolyze cellulose into glucose in a similar fashion. However, although dilute sulfuric acid can recover sugars from hemicellulose with high yields, yields from dilute acid hydrolysis of cellulose are much lower because higher temperatures are needed to overcome its high crystallinity, resulting in glucose degradation (Grohmann et al. 1985; McMillan 1994; Hsu 1996). Because of their high selectivity, enzymes realize the high yields important to competitiveness (Wyman 2007). Concentrated acids can also realize high yields, but costs to recover the high loadings of acid needed are high (Hsu 1996; Yang and Wyman 2008).

Because enzymes cannot penetrate the complex structure of most types of biomass well, a pretreatment step is essential to high yields, and one approach is to employ dilute sulfuric acid to remove much of the hemicellulose and open up the structure for effective sugar release from cellulose by enzymes. In this case, temperatures of about 140–180°C can be employed at acid concentrations of about 0.5%–2.0% and residence times on the order of 10–30 minutes to recover about 80%–90% of the sugars in hemicellulose during pretreatment plus some of the glucose from cellulose. The resulting insoluble solids are enriched in cellulose and lignin, and a small fraction can be used to support growth of the fungus *Trichoderma reesei* or other aerobic organism that produces enzymes known as cellulase to depolymerize cellulose into glucose and hemicelluloses to break down hemicellulose not removed by pretreatment into its component sugars. These enzymes are then added to the pretreated solids to release most of the sugars left in the solids, and an organism can be added to the same vessel to ferment the sugars released to ethanol in a configuration known as the simultaneous saccharification and fermentation (SSF) process, an approach that enhances rates, yields, and concentrations by reducing inhibition by the sugars released and also lowers containment costs (Spindler et al. 1991; Wyman et al. 1992; Katzen and Fowler 1994; Ingram and Zhou 2002). Sugars released in pretreatment, mostly from hemicellulose, are fermented to ethanol with an organism that has been genetically modified to achieve high yields from the five carbon sugars arabinose and xylose that native organisms could not effectively ferment to ethanol (Ho and Tsao 1995; Zhang et al. 1995; Ingram et al. 1997). The hemicellulose sugar stream could also be left with the pretreated solids and fermented to ethanol in the same vessel in a configuration known as simultaneous saccharification and co-fermentation (SSCF). The broth from fermentation is sent to a distillation and dehydration system to remove the ethanol

while leaving the unconverted solids (mostly lignin), water, and other leftovers in the column bottoms. Contrary to many incorrect statements, water is not removed from the ethanol, and ethanol recovery is not very energy intensive in a well-engineered process. Rather, we can take advantage of the high volatility of ethanol compared to water to remove high-purity ethanol from a messy fermentation broth and concentrate and burn the residues left in the water to provide more than enough energy to meet all the heat and power needs for the process with significant amounts of electricity left over to export (Wooley et al. 1999a; Wyman 1999b, 2007).

The pathway outlined above represents the configuration often considered currently. However, as will be discussed later, enzymes are very expensive and are the major showstopper to commercialization. In particular, enzymes suffer from two limitations: (1) they are expensive to make due to the aerobic fermentations used and (2) large doses of enzyme are required to produce sugars with adequate yields (Lynd et al. 1996, 2002; Aden et al. 2002). An alternate configuration is to employ a single organism or consortium of organisms that can both make enzymes and ferment the sugars they produce to ethanol. Several advantages result from this approach. First, less equipment is needed, and fewer transfers are required. In addition, the fermentative organisms are anaerobes, thereby avoiding the high power requirements for making enzymes using fungal systems such as *T. reesei*. This consolidated bioprocessing (CBP) approach could thus lower costs by reducing capital investments and energy costs. However, although native fermentative organisms can produce enzymes anaerobically, they have a low selectivity for ethanol, resulting in too low yields, and several groups are now working to eliminate competitive pathways so that high yields of ethanol are achieved (Lynd et al. 2005). In addition, these organisms must be hardened to withstand an industrial environment and realize high ethanol concentrations.

Many other pretreatment approaches have been trialed over the years other than dilute acid, and a few such as ammonia fiber expansion (AFEX), controlled pH, ammonia recycle percolation (ARP), lime, and sulfur dioxide technologies can be effective (Mosier et al. 2005b). Those at low pH such as use of sulfur dioxide remove hemicelluloses in the same manner as outlined above, with the primary difference being in the ability to recover and recycle the sulfur dioxide (Schell et al. 1991). Such low pH pretreatments also produce predominately monomeric sugars that many organisms can ferment to ethanol (Wyman et al. 2007). Use of only water or addition of buffers to maintain the pH nearer to neutral will preserve most of the sugars as short chains that dissolve in water, with the goal of reducing their degradation (Mosier et al. 2005a). On the other hand, the overall yields of sugars and oligomers are somewhat lower than for dilute acids, and either organisms must be used that can ferment the soluble oligomer chains or additional steps are needed to break them down into fermentable monomers (Eggeman and Elander 2005). Pretreatment through addition of a base such as lime or sodium hydroxide opens up the cellulose to enzymes by removing lignin but can take longer to react or cost more (McMillan 1994). Ammonia can also be employed to lower pH, but its release for recovery and recycle when the pressure is dropped following pretreatment results in no visible removal of lignin or hemicelluloses (Dale et al. 1996). Yet the resulting solids can be highly susceptible to sugar release by enzymes, and the product stream does not form strong inhibitors of fermentation or enzymatic hydrolysis (Wyman et al. 2007). Much more experience has been developed with dilute sulfuric acid than for the other options because of historical limits in funding for such research, but the others can have important advantages and synergies that should be explored.

Environmental Considerations

Agricultural and forestry residues and organic portions of municipal solid waste can have a negative impact on the environment as they decay. On the other hand, converting these residues to ethanol can offer immediate and sustained GHG advantages and simultaneously enhance domestic fuel production (Paustian et al. 1998; Tilman et al. 2001; DiPardo 2002; Wyman 2003b; Zhang et al. 2007; BR&Di 2008; Fargione et al. 2008; Smith et al. 2008). In general, cellulosic ethanol can be a low carbon fuel and provide a valuable replacement for gasoline from petroleum. Although production and combustion of ethanol adds CO₂ to the atmosphere, an equivalent amount of CO₂ can be taken up as the next rotation of agriculture feedstocks is grown to replace that used to produce ethanol (Wyman 2003a). Thus, cellulosic ethanol provides an opportunity to recycle carbon instead of continually building up carbon in the atmosphere as fossil fuels do, and it has been estimated that ethanol from corn stover could reduce GHG emissions by over 80% compared to petroleum-derived fuels (Wang et al. 1999). Exporting the excess power produced by burning lignin and other portions of cellulosic biomass not utilized for making ethanol can reduce the amount of coal used to produce electricity in the grid, potentially resulting in negative emissions of CO₂ compared to the status quo (Wyman 1994a). Moreover, because the large amount of virtually pure CO₂ (around 300 kg CO₂ per dry ton of corn stover) produced during fermentation could be sequestered more easily than being considered for capturing CO₂ from burning coal, ethanol could actually become a negative GHG emission fuel. Because these agricultural residues are associated with food production, they will be grown whether we use them for making fuel or not, largely avoiding many of the concerns about indirect land use now being hotly debated (EPA 2005; BR&Di 2008; Fargione et al. 2008).

Even if GHG benefits are demonstrable, other sustainability and environmental considerations must be addressed for use of agricultural residues. In particular, removing agricultural residues from the land can impact soil cultivation and the needs for fertilizer, pesticides, and other chemicals, all of which can impact soil, water quality, air quality, site productivity, and GHG emissions (Kim and Dale 2004; EPA 2005; Smith et al. 2008). Some residues such as sugarcane bagasse, rice hulls, rice straw, and corn fiber that are typically removed from the field anyway can be employed without additional negative consequences when properly managed, and their use results in little, if any, net additional demands for cropland, fertilizer, pesticides, or water. On the other hand, leaving corn stover, wheat straw, and other plant matter remaining after food is harvested on the field helps maintain soil organic and inorganic matter and protect against erosion (Lal 2006). The amount of sustainably harvestable residues varies with location and depends upon climate, soil texture, rain fall, and the production practice used (Tilman et al. 2002; BR&Di 2008). For example, conventional till production of corn leaves more residues in the field than no-till systems (Kim and Dale 2005; BR&Di 2008). However, estimates of the amounts of residues that can be removed sustainably are still being refined.

Availability and Composition of Agricultural Residues

In the last century, innovation in various disciplines, such as plant breeding, crop protection, soil fertility, and plant nutrition have supported an enormous increase in agricultural

productivity (Sambamurty 2002; Tilman et al. 2002; Jauhar 2006; Wenzel 2006). As a result, the United States has large amounts of (1) crop residues, (2) agricultural processing residues, (3) animal manures and other wastes, and (4) grasses. Feedstock compositions directly affect product yield and tech-economical feasibility of ethanol conversion process and vary significantly among different kinds of agricultural residues (Table 9.1) (Wyman 2007). As shown in the second through eighth columns of Table 9.1, the high carbohydrate content (i.e., cellulose and hemicellulose) of many crop residues, such as corn stover, result in high theoretical ethanol yields, making them attractive candidates for fuel ethanol production. Among these, corn production residues, such as corncobs and corn stover, cotton processing residues, and sugarcane bagasse contain relatively high fractions of carbohydrates and relatively low lignin, making them particularly amenable for making fermentable sugars. On the other hand, nutshells are not promising feedstocks for bio-conversion to fuel ethanol due to their high lignin content and resulting low amounts of carbohydrates.

Competition for feedstocks and harvesting and transport costs are critical, particularly for initial commercial ventures. For example, because the stalks left after extraction of sugar from sugarcane are already at a central location, no additional costs are incurred for collection and transport. However, these materials have value as a fuel for generating process heat and possibly electricity, which still must be taken into consideration. Corn fiber is also attractive because of its availability at a processing facility, but it has value as a binder and source of protein for cattle feed. Because residues such as corn stover or just the stalks are left on the field after harvesting the kernels, additional costs are incurred to gather and transport these materials compared to bagasse or corn fiber, and such residues frequently have value as a soil stabilizer and nutrient source, with the result that some must be left in the field (Karlen et al. 1984; Randall et al. 2006; Hoskinson et al. 2007). Other feedstocks such as rice straw are of interest because they are burned following rice harvest to prevent spread of plant diseases, making them potentially available. However, additional costs are incurred to harvest and transport such materials to a central processing site, and the high amounts of silica have a large impact on sugar and resulting ethanol yields and complicate processing to ethanol.

Table 9.2 summarizes the production of various categories of agricultural residues potentially available for conversion to ethanol and other products by 2030. Figure 9.1 breaks these totals down to show the current and predicted availability of those feedstocks with greatest potential impact in the United States (Perlack et al. 2005). The amount of available feedstock is the residue that can be sustainably removed from the field, which is less than the total produced. The sustainably removable amounts depend on various factors, such as the annual crop residue collection technology, equipment used, soil type, climate, and crop tillage practices (Blanco-Canqui et al. 2006; Hoskinson et al. 2007). The predicted feedstock availabilities listed are based on two different scenarios: a relatively conservative assumption of moderate crop yield increases without land use changes to accommodate perennial crops (energy crops) and a high-end assumption that crop yields increase significantly with land use change to accommodate energy crops. As the data show, corn stover ranks first by a large margin in terms of availability in all scenarios. Yet various crop residues can play an important role for fuel ethanol production, particularly when they are combined with others. As crop yields increase with land use change, the availability of some feedstocks, such as soybean straw and sorghum, could increase significantly.

Table 9.1. Major components contained in leading agricultural residues.

Feedstock	Arabinan	Xylan	Mannan	Galactan	Glucan	Cellulose	Hemicellulose	Extractives	Lignin	Ash	Reference
Crop residues											
Corn stover	3.5	21.4	1.8	2.5	36.1	36.1			17.2	7.1	Wyman et al. 2005a
Cotton straw					42	42	12		15		Ladisch 1989
Rice straw		24.5			34.2			17.9	11.9	16.1	Wiseloge et al. 1996a
Barley straw	3	24.2		0.4	40.5	40.5			19.8	3.8	Linde et al. 2006
Rye (<i>Secale cereale</i>)	2.47	19.46		0.31	33.12	33.12			19.8	6.15	Pimentel and Patzek 2005
Wheat straw											
Oat straw	2.5	21.2	0.3	0.7	38.2	38.2		13	23.4	10.3	Wiseloge et al. 1996b
Sorghum, fiber					39.4	39.4	27.1		17.5		Garrote et al. 1999
Lentil (<i>Lens culinaris</i>)					41.85	41.85	27.21		7.82		Dolciotti et al. 1998
straw					38.5	38.5	16.3	1	11.5		Lopez et al. 2005
Soybean, stems											
Bean											
(<i>Phaseolus vulgaris</i>)											
straw											
Chickpea, whole straw											
Banana stem											
Pineapple leaf											
Sunflower stalks											
Tobacco stalk											

Table 9.1. Continued.

Feedstock	Arabinan	Xylan	Mannan	Galactan	Glucan	Cellulose	Hemicellulose	Extractives	Lignin	Ash	Reference
Processing residues											
Corn cobs						45	35	5	15		Koch 2006
Corn fiber	10.0	19.0		4.0	30.0	35.3			8		Allen et al. 2001a
Corn husk		43.2				21.8			10.9		Kurakake et al. 2001
Cotton linter, seed hull						85-90	1-3		0.7-1.6	0.8-2	Han 1998
Sugarcane bagasse	1.6	23.11	0.3	0.45	43.39	43.39	25.46	1.52	24.09	2.84	
Rice hull	1.9	14.4	0	1.1	31.2	30.9	16.8		35.9		Laser et al. 2002
Oat hull	1.2	15.5	0	0.0	48.9	48.4	16.1		16.2		Antal et al. 2000
Almond shells	0	26.1	0	1.8	25.0	24.7	27		27.2		Antal et al. 2000
Walnut shell	0	17.2	0	2.2	21.2	21.0	18.8		32.7		Antal et al. 2000
Pecan shell	0	2.8	0	1.1	5.6	5.6	3.8		70		Antal et al. 2000
Carrot peelings						39.49				9.71	Li et al. 2007
Coconut fiber	0.1	17	0.1	0.4	34.9	34.9	17.5		33.5		Han 1998
Coconut shell	0	25.5	0	0.0	24.5	24.2	24.7		34.9		Antal et al. 2000
Oil-palm empty fruit bunch						35.71		5.67	21.97	6.82	Minowa et al. 1998
Olive husk						24	23.6	9.4	48.4	3.45	Demirbas 1997
Orange peel						9.91			8.7		Grohmann et al. 1995
Manure											
Cow manure						32.6	23.8		26.8	43.7	Stiller et al. 1996
Horse manure						37.8	32.4		19.6	11.2	Stiller et al. 1996
Broiler litter						18.1	18.05		2.82		Rasool et al. 1996

Table 9.2. Potential availability of agricultural resources in the United States by 2030 (Perlack et al. 2005).

Resource	Million Dry Tons
Crop residues	446
Animal manures and residues	44
Agricultural processing residues	44
Total	534

Experience with Biological Conversion of Agricultural Residues

Numerous studies have reported conversion of various agricultural residues and processing wastes to sugars and to ethanol using a variety of pretreatment and fermentation technologies. As shown in Table 9.3, high overall yields of xylose, glucose, and other sugars have been realized through pretreatment followed by enzymatic hydrolysis for the following feedstocks: corn stover, barley straw, and corn fiber. On the other hand, much lower yields have been reported for flatpea hay, wheat straw, and sugarcane bagasse. Unfortunately, it is challenging to compare many of these results accurately due to changes in methods, enzyme formulations, analytical approaches, and data reporting. However, one team did systematically investigate bioconversion of corn stover, which has the highest availability in the United States (see Figure 9.1), for different pretreatment operations in combination with the same enzymes and using the same methods (Wyman et al. 2005a). In this case, these leading pretreatments that spanned pH values from about 1.2–11 realized over 90% overall yields of xylose and glucose. Some work has also been conducted in determining the fermentability of the sugars released to ethanol as it is vital to obtain high ethanol yields in the fermentation step (Wyman et al. 2005b). In this case, inhibitory effects of compounds produced or released during up-stream processing by operations such as pretreatment must be avoided or overcome. In addition, high ethanol yields must be realized from all pentose and hexose sugars that tend to be prevalent in agricultural residues. Furthermore, some pretreatments such as controlled pH produce substantial amounts of oligomeric sugars that many organisms cannot ferment to ethanol directly, and steps must be introduced to either hydrolyze these to monomers and possibly dimmers, or organisms must be employed that can fully utilize these soluble polymers (Yang and Wyman 2008).

Harvesting and Transport of Agricultural Residues

As one approach to harvesting, collection, and transport of corn stover, a simple two-step operation of baling and bale collection and delivery to the processor was identified to replace the former five-step procedure of raking, baling, field loading, hauling, and unloading (www.ceassist.com 2000). By turning off the spreader on the corn combine, a windrow was left behind that could be more easily baled, resulting in a collection of about 1.5–2.0 dry tons per acre of stover that could be increased to 2.5–3.5 dry tons per acre by adding a rake in front of the baler. Both round and square bales were collected with a target of 1200 dry lb per round bale and large rectangular (4' × 4' × 8') bales and 650 dry tons

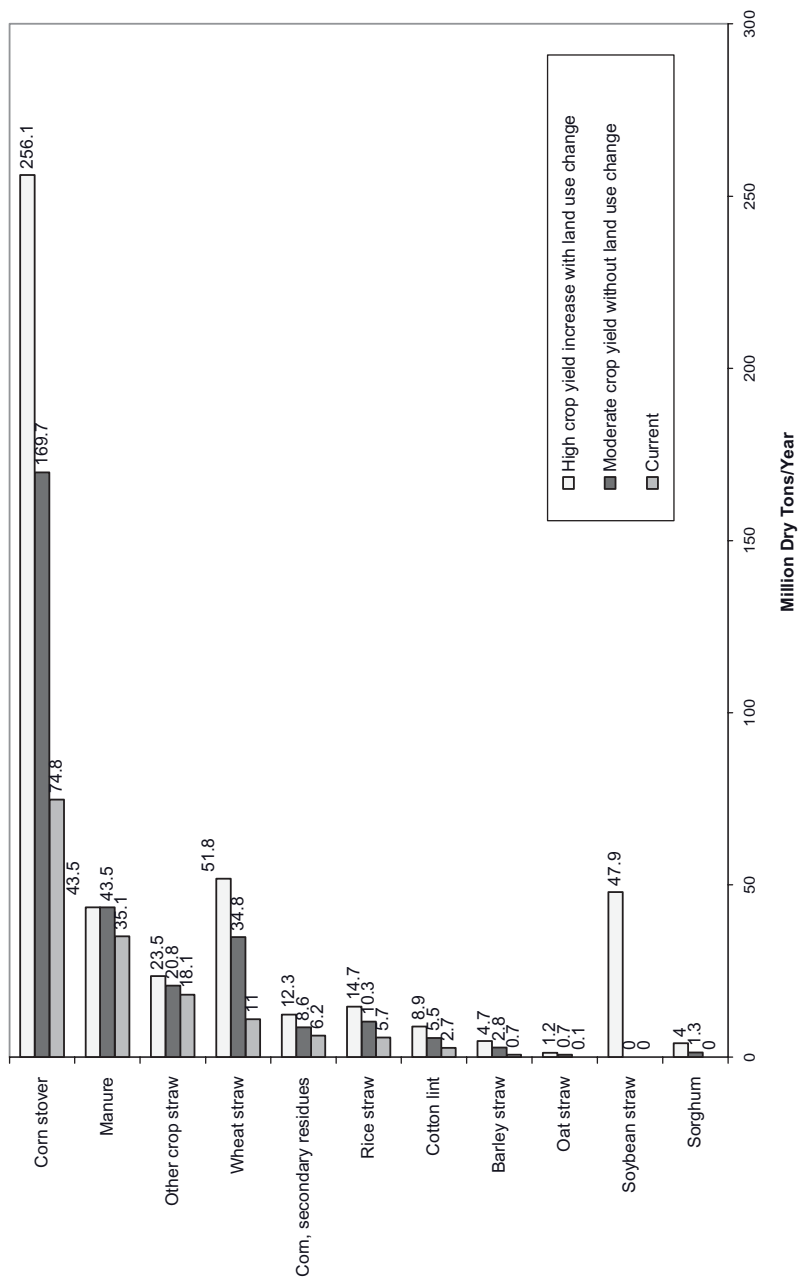


Figure 9.1. Current and projected availability of agricultural wastes in the United States.

Table 9.3. Pretreatment and enzymatic hydrolysis of various agriculture residues.

Feedstock	Pretreatment		Enzymatic hydrolysis			Overall yield, % #		
	Method	Xylan yield ^b %	Cellulase loading, FPU/ g cellulose	Conversion %	Xylan	Glucan	Sugar	Reference
Agricultural residues Barley straw	Steam-H ₂ SO ₄ explosion, 0.2% acid, 200°C, 5 minutes							Linde et al. 2006
	Water, 179°C, 23 minutes	55.6						Nabarlatz et al. 2007
	Water, 60/180/195°C, 15/10/3 minutes		13.4	50				Rosgaard et al. 2007
Corn stover	Acid H ₂ SO ₄ 0.49%, 160°C, 20 minutes	85.1	15					Wyman et al. 2005a
	Steam-SO ₂ explosion, 3% SO ₂ , 190°C, 5 minutes		15	108				Oehgren et al. 2007
	Water flowthrough, 200°C, 24 minutes, 10mL/min	96.3	15	98.7				Wyman et al. 2005a
	Steam explosion, 190°C, 5 minutes		15	103				Oehgren et al. 2007
	AFEX, 90°C, 5 minutes		15	96				Wyman et al. 2005a
Flatpea hay	ARP, 15% ammonia, 170°C, 10 minutes	47.2	15	90				Wyman et al. 2005a
	Control pH, 190°C, 15 minutes	57.8	15	90				Wyman et al. 2005a
	Lime, 55°C, 4 weeks	24.4	15	93				Wyman et al. 2005a
	Acid H ₂ SO ₄ 0.55%, 180°C, 10–20 minutes		42	80				Torget et al. 1992
Wheat straw	Acid H ₂ SO ₄ 0.7%, 160°C		33	92				Grohmann et al. 1986
Agricultural processing wastes								
	Almond shells							
	Steam water	90						Montane et al. 1993
	Water, 179°C, 23 minutes	64.8						Nabarlatz et al. 2007
	Water, 179°C, 23 minutes	73						Nabarlatz et al. 2007
	Water, 215°C, 2 minutes	52.6	15	86	82	99		Allen et al. 2001b
	Water, 179°C, 23 minutes	52.5						Nabarlatz et al. 2007
	Steam, 220°C, 2 minutes	48	15	85				Laser et al. 2002
Sugarcane bagasse	Water, 220°C, 2 minutes	91	15	75				Laser et al. 2002
	Steam explosion, 200°C, 10 minutes		20 ^a	95				Dekker and Wallis 1983

^aCellulase loading unit FPU/g dry weight substrate.

^bTotal xylan, glucan, and sugar yield based on original component content, including oligomeric and monomeric sugars.

for intermediate-sized square bales. Round bales were rapped with three layers of plastic net to ensure they did not break apart when collected and handled later in processing. The bales were picked up at speeds of 5–7 mi/h in the field by a tractor-loading arm operated by one person capable of picking up bales in any orientation, rotating them into the proper position, and loading them onto a “load and go” trailer that holds at least 17 round bales and folds to legal width when empty. Some haulers completed a loading cycle of about 21,000 dry lb (17 round bales) in less than 20 minutes. The unit could safely traverse corn fields, including crossing ditches, and still travel at highway speeds of up to 60 mph for transport to the collection center where the load could be weighed, sampled for moisture, and unloaded in less than 10 minutes. The trailer could then return to the field for another load. Overall, this approach was said to be able to cut corn stover costs by up to nearly half. However, a potential concern is the likelihood of picking up large amounts of dirt and rocks when the material is taken from the field piles.

Various companies have collected corn stover, and particularly corncobs, and it is important to determine if these collection methods would work for making biofuels. For example, QO Chemical in conjunction with the Anderson Brothers of Columbus, Ohio, ran a “Cob Saver Program” in the late 1980s to collect corncobs in addition to the kernels from the field. This entailed substituting a sieve plate with larger diameter holes into combines so that the cobs would pass through the holes during harvesting rather than just the kernels; the stalks and husk passed over the plate and were returned to the field. The cobs were easily separated from the kernels when the truck was unloaded and stored in piles for future conversion to furfural and other products. This system was extensively tested in the field, and excellent cob recovery was demonstrated with little damage to the kernels. It also avoided gathering feedstock off the ground after harvesting and the inevitable contamination with dirt and rocks that increase transportation costs for nonusable materials and can damage processing equipment.

Although the focus above was on corn stover and cobs because of the size of the resource and relevant experience, other feedstocks could be used for the commercial process. Because most Ag residues, herbaceous materials, and hardwoods behave similarly in pretreatment and fermentation systems with small adjustments in chemical (e.g., sulfuric acid) additions and residence times, it should not be a major issue to employ wheat straw, switchgrass, or other biomass sources abundant in the local processing area (Torget et al. 1992). Important considerations impacting feedstock flexibility can be such seemingly simple aspects as biomass conveying because the details of its design vary with bulk packing density and other feedstock properties, and failure to recognize these important distinctions can cripple a project and result in financial collapse (Wiltsee and Bain 2000).

Storage and Logistics

Agricultural residues are only available for a limited time frame each year, but processing facilities must be run around the clock for virtually the entire year to have any hope of realizing reasonable returns on the huge investments required. This requirement is particularly critical for production of commodity products as margins are thin. Thus, a vital consideration is how to ensure a year-round supply, and only two options are apparent: (1) store enough material during the harvest season to last the entire year or (2) have access to a number of feedstocks that can be harvested year round. The latter may be a viable strategy but is very

regional in nature. Thus, our focus will be to discuss considerations in storing agricultural residues.

Given the low margins and high capital costs, it is highly desirable to store agricultural residues in the simplest and least costly manner possible, and simply piling up biomass is about as simple a way as there is. For example, sugarcane bagasse has been stored in piles that are properly maintained to prevent moisture accumulation. Tractor trailer rigs drove on to large piles of bagasse at a furfural plant in Belle Glade, Florida over a period of about 30 years to dump their loads, and bulldozers then spread the material around so the surface sloped downward. Driving large equipment on the piles compacted the bagasse, thereby making it nearly impenetrable to water and also difficult for the pile to catch fire. Slopping the piles not only allowed trucks and bulldozers to travel on the pile but ensured that water would run off, thereby keeping the moisture levels reasonably constant at about 50%. The result was little degradation of the hemicellulose or cellulose in bagasse.

In a systematic study, samples taken from various depths in a pile of sugarcane bagasse over a period of 3–26 weeks were analyzed by analytical pyrolysis (Agblevor et al. 1994). It was found that samples taken from the center of the piles after 3.25, 6.5, 13, and 26 weeks of storage had very small changes in the distribution of hemicellulose, cellulose, and lignin, while hemicellulose and cellulose contents dropped while lignin increased for samples taken from the regions near the outer surface layer. They also found that pentosans were degraded more rapidly than hexosans in the outer regions in the pile where microbial activity was high, consistent with experience that hemicellulose is more readily decomposed than cellulose which is in turn more susceptible to attack than lignin. Thus, these results demonstrate that deterioration only occurred in a very shallow layer near the surface of the piles but not in the bulk of the material, consistent with industrial experience with large piles. If such an approach would work as well for corn stover and other agricultural residues, it would greatly simplify storage.

It has been shown that wrapped bales of corn stover can be stored for extended times with little degradation, consistent with experience with sugarcane bagasse. All bales were stored in the open, with high moisture bales and uncovered square bales processed first while the normal moisture round bales, protected from the weather by their plastic wrap, could be processed later. Open-field storage of wrapped round bales is common practice for straw, hay, and similar materials, with open fields of such bales a common sight in Vermont and New Hampshire, as well as Nebraska, Iowa, and other parts of the Midwest. Corncobs have been compacted for long-term storage for furfural production over more than a year, similar to the approach taken for bagasse described above. However, it is unclear if the lower bulk packing density of corn stover and other agricultural residues will allow this approach.

Another consideration is whether to store biomass near the source or near the conversion facility. The former simplifies gathering operations and avoids moving material that could degrade during storage. However, it is simpler to manage a few piles of biomass than to try to ensure numerous piles are properly cared for to minimize deterioration.

Economic Considerations and Barriers

A view of the economics of converting cellulosic biomass to ethanol can provide a useful context for understanding the impact of feedstock cost and availability on competitiveness. However, it is vital to keep in mind that no cellulosic ethanol plant has yet been built, and

although various cost estimates have been published, all such analyses are just estimates (Wooley et al. 1999a; Aden et al. 2002). Thus, such information is primarily useful to help understand key cost drivers that can have a strong influence on costs, but accurate information will not be possible until several operational plants have accumulated enough of a learning curve to be reaching technology maturity. In addition, costs are highly dependent on the technology chosen for the design, and many low-cost approaches are not accessible as they are often protected as trade secrets and know-how. For this reason, this chapter will provide a basic outline of processing costs, and the reader is referred to other publications for detailed process designs and estimates while keeping in mind the approximate nature and other limitations of all of such analyses (U.S. DOE 1993; Wooley et al. 1999a,b; Aden et al. 2002).

A facility for processing a nominal 2000 dry tons per day of corn stover or 666,666 dry tons annually based on typical on-stream times is taken here to provide a perspective on the economics of converting agricultural residues into ethanol. In general terms, the process is based on use of dilute sulfuric acid for pretreatment and other features as outlined early in this chapter. Furthermore, the composition of corn stover is based on that reported in a coordinated study by the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI), as summarized in Table 9.4 (Wyman et al. 2005b). This table also includes the maximum amount of ethanol that could be produced from these sugars at the theoretical limit.

Operating conditions for this analysis are as developed by Lloyd and Wyman as part of the CAFI study to achieve the highest total yields of glucose plus xylose from corn stover in a coordinated comparison to performance with other pretreatment options (Lloyd and Wyman 2005). For this approach, Table 9.5 outlines the operating conditions employed experimentally and the corresponding sugar yields. It is further assumed that these sugars can be fermented to ethanol with yields of about 92% of the theoretical maximum based on experience in industry and with the recombinant organisms employed for fermenting the five carbon sugars arabinose and xylose as well as galactose and mannose in addition to fermentation of glucose, and that 99.9% of the ethanol can be recovered in distillation and dehydration using proven technology. The lignin is burned to generate heat and power, and any extra left after heating streams and providing power in the process is exported for sale.

Table 9.4. Corn stover composition and corresponding maximum potential ethanol yields (Wyman et al. 2005b).

Component	%	Lb etoh/ton	Gals/ton
Glucan	36.1	410.025	62.12495
Xylan	21.4	248.586	37.66452
Arabinan	3.5	40.657	6.160086
Mannan	1.8	20.444	3.097643
Galactan	2.5	28.395	4.302282
Lignin	17.2		
Protein	4.0		
Acetyl	3.2		
Ash	7.1		
Uronic acid	3.6		
Frees sugars	1.2		
Other	-1.6		
Total	100.0	748.107	113.349

Table 9.5. Operating parameters and sugar yields for pretreatment and enzymatic hydrolysis of corn stover (Lloyd and Wyman 2005).

Parameter	Based on Reference	Improved Performance
Pretreatment		
Sulfuric acid concentration	0.5%	0.0%
Temperature	160°C	140°C
Reaction time	20 minutes	60 minutes
Hemicellulose sugar yield	85.1%	90%
Glucose yield	6.3%	7.0%
Enzymatic hydrolysis		
Enzyme loading	15 FPU/g glucan	NA
Temperature	50°C	NA
Reaction time	6 days	NA
Hemicellulose sugar yield	8.5%	8.0%
Glucose yield	85.4%	90.0%
Overall ethanol yield calculated from above	89.7 gal/dry ton	99.4 gal/ton

Table 9.6. Raw material costs and unit costs for different yields scenarios.

Element	Yield, gal/ton Cost, \$/ton	99.4 per gallon	89.7 per gallon	82.6 per gallon	73.6 per gallon
Feedstock	60	0.6035	0.6690	0.7266	0.8152
Sulfuric acid	200	0.0000	0.0170	0.0185	0.0208
Lime	70	0.0000	0.0089	0.0097	0.0109
Cellulase	0	0.0000	0.0000	0.0000	0.0000
Nutrients	70	0.0646	0.0707	0.0710	0.0712
Total		0.6680	0.7656	0.8258	0.9181
Labor		0.0175	0.0194	0.0210	0.0236
Total with labor		0.6855	0.7849	0.8468	0.9417
Electricity sales	\$0.05/kwh	(0.0391)	(0.0510)	(0.0744)	(0.1100)
Total with electricity		0.6464	0.7340	0.7724	0.8317

From this information, cash costs were estimated based on the assumed costs for feedstock, sulfuric acid, lime, nutrients, and labor outlined in Table 9.6. Enzyme costs were not included at this point because of the uncertainty in these values and will be considered later. Based on the yields reported by Lloyd and Wyman, the total estimated cost is about \$0.785/gal prior to subtracting any coproduct credit for exported power. Because others have reported much lower yields based on less optimal performance data, a breakdown of operating costs are also included for lower overall ethanol yields of 82.6 and 73.6 gal/dry ton to give total costs estimates of \$0.847 and \$0.942/gal, respectively.

These estimates clearly show the importance of biomass costs in the economics as they dominate the overall cash costs. Thus, it is highly desirable to seek low-cost agricultural residues that can dramatically cut these costs. However, several other points must be kept in mind for these rough estimates. First, they are at the plant gate and do not include transportation, taxes, marketing, and a myriad of other costs that must be adsorbed before the fuel

reaches the consumer. In addition, up to 50% of these totals could be added to compensate for the fact that ethanol contains about two-thirds the energy content of gasoline; however, ethanol can also be used more efficiently than gasoline in a properly optimized engine, which can make up for up to 50% of this difference (Bailey 1996). Although benefits are factored into the labor costs as 30% of wages and plant supervision and management are included, these costs do not include other overhead or manufacturing costs such as maintenance and administration or other costs that are typically factored off of capital estimates. First plants may also require more labor than assumed here.

A rough estimate of the income from sale of electricity is included in Table 9.6 based on a selling price of \$0.05/kwh for power. In this analysis, about a third of the heat generated by burning residues was calculated to be left for generating electricity at an assumed efficiency of 33%, that is, 1 Btu of electricity is assumed to be produced for every 3 Btu of heat available. These calculations take a penalty for the water in the lignin and other residuals by assuming somewhat more than half is water that must be vaporized during combustion. Furthermore, these values do not address the amount of electricity needed to run equipment because of the detail required for such estimates, but they also do not include any possibility of heat exchange or cascading to substantially reduce the amount of heat needed to bring the biomass feed up to pretreatment temperature or heat up the large fermentation beer stream to boiling for distillation. Thus, these values should be regarded as providing a rough idea of how much revenue could be gained from selling power, with the upper bound being about three times the value given and the lower bound being zero, the latter corresponding to either no power left to sell or no market into which to sell it. Of course, these values should be scaled proportionately if a higher or lower selling price is assumed for electricity.

Table 9.6 also includes a more mature performance case that obtains better yields for each step in the process, as outlined in Table 9.5. Now, a cost of about \$0.686/gal of ethanol is calculated for a feedstock cost of \$60/dry ton, as outlined in this table. This estimate would drop to on the order of \$0.64/gal after subtracting for sale of coproduct power, again subject to all of the caveats described above for other costs, capital recovery, and sale of power. Overall, the estimates show that high yields are beneficial, but that sale of power can somewhat compensate by gaining value from the unutilized fraction. In addition, even the costs without including power and for low yields could be competitive with gasoline and ethanol from corn.

The cost estimates above do not include repaying for capital or the interest on debt to obtain that capital. However, estimating capital costs is extremely challenging, and as a result, most values can only be used as a rough guideline as to what to expect. For that reason, we will employ a guesstimate of \$4.00/annual gallon for the 2000 tons/day base case to give a rough idea of the investment level required. This value is in line with estimates developed by NREL and others (Eggeman and Elander 2005; Wooley et al. 1999a). It is also consistent with the idea that a cellulosic ethanol facility combines the capital needs of a corn ethanol plant to make sugars and ferment them to ethanol with the capital demands of building a biomass power plant to burn the residual lignin and other unutilized portions of biomass to produce heat and power for the process with excess to export. In addition, we expect the ethanol facility to be somewhat more expensive than for a corn ethanol process because of the harsher conditions required for pretreatment and the longer times and more dilute solutions for sugar fermentation. Thus, about \$4.00/gal appears in the right range. Yet, first projects can be more expensive than this estimate because of concerns about inexperience with the technology and resulting overdesign to ensure the project is successful. Learning curve experience will rapidly

lower the cost once a facility is running through greater throughput with the extra equipment and improvements in operating conditions and biological catalysts (Goldemberg et al. 1993; Moreira and Goldemberg 1999).

Another aspect of capital costs is that they do not change linearly with scale of operation but scale as a fractional power of size. For example, employing the exponent of 0.67 often used as the norm would result in the capital cost increases by about 59% when the size of the operation is doubled instead of being 100% more expensive. Such economies of scale can be understood from the perspective that amounts of material and fabrication labor are proportional to surface area while capacity is proportional to volume of an equipment item. Furthermore, the surface to volume ratio of equipment drops with increasing size and does not increase linearly. Exponential scale factors vary with the type of equipment, as tabulated in several reference books and are almost always less than 1.0 (Peters et al. 2003). The result is that unit capital costs drop as the process throughput increases, leading engineers to favor larger-scale operations to minimize capital costs. Consequently, the assumed cost of \$4.00/annual gallon for cellulosic ethanol would drop to about \$3.18/annual gallon if the capacity was doubled to 4000 tons/day and the 0.67 exponent were applicable.

To counter the possibility of lower unit costs for larger facilities, it is often stated that economies of scale cannot be realized in biomass processing because of the low density of biomass crops. However, if we consider that, about 3.75 dry tons of corn stover/acre would result for a corn productivity of 150 bushels/acre given the virtual one-to-one ratio of above-ground plant to corn kernels. In addition, within the 50-mile radius typically considered to be reasonable for collecting corn and wood, this volume of corn stover production would amount to about 1.875 billion gallons of ethanol. Even if we assume that only 1.0 dry tons/acre on average can be accessed and/or removed sustainably from the field, the result would be about 500 million gallons of ethanol annually within the 50-mile radius. A few studies have clearly shown that even this low harvest rate favors conversion facilities of at least 10,000 dry tons per day, giving an annual ethanol production of about 250 million gallons (Wyman 1995). The primary limitation to building plants of this size is the high capital costs; for example, a 10,000 gallon per year operation would cost on the order of \$3.0 billion or more. Although it may be possible to raise such large sums for a mature process, the risk of first applications is considered too great for most investors, and it is extremely unlikely that first projects would be this large.

A key question is how to annualize up-front capital costs over the life of the facility, and a useful approach is based on a projected cash flow coupled with an appropriate discounting formula (Wyman 1995; Wooley et al. 1999a). However, the challenge is the choice of appropriate parameters in this analysis which are in turn tied to many other factors such as economic lifetime of the plant and expected rate of return by the financing entity. Furthermore, the interest rate and economic lifetime will depend on the stage of technology development as high rates of return are expected for first applications while much lower rates can be negotiated once a successful track record is firmly established. Thus, a first project may require payout in only a few years time while mature technology may be able to pay off capital over a period of 20–30 years. Unfortunately, this classical relationship between rate of return and risk presents a major chicken-and-egg dilemma for building first-of-a-kind cellulosic ethanol facilities in that coupling high rates of return demanded by investors with a capital-heavy design to ensure successful operation will almost surely result in overall costs that are too high for the commodity fuel market. In this regard, it is vital to remember that the competition is gasoline which has benefitted from over a hundred years of learning curve advances, paid off capital, substantial subsidies to ensure stable supplies from hostile regions of the

world, an established infrastructure, and no consideration for societal costs associated with its use.

Return on capital could result in virtually a 100% capital charge in the first year for new technology from investors demanding fast paybacks to as low as 10%–15% if project finance could be used similar to classical utility financing. Given that fuels are a commodity business, it is unrealistic to expect very rapid payback times. If we take a 20% annual charge as likely for reasonably mature technology, the total of cash cost plus capital charges would amount to something like \$1.60/gal for the case based on laboratory data in Table 9.5 and a capital cost of \$4.00/annual gallon. To this, additional costs need to be added for maintenance, overhead, and the other aspects mentioned before that were not included. The resulting cost could be promising compared to gasoline when oil prices are high but not when they are at lower levels. However, given their large contribution to the estimated costs, use of a low-cost residue could improve competitiveness significantly.

A key element left out of these estimates is the cost of enzyme. In many studies on enzymatic hydrolysis of pretreated biomass, enzyme loadings of about 15 FPU/g glucan were applied to realize good sugar yields from the pretreated solids. However, at a typical specific activity of about 0.5 FPU/mg protein, these loadings amount to on the order of 0.25 lb of protein/gallon of ethanol produced including the ethanol made from the hemicellulose sugars that are often released during pretreatment. Reports have been made of advances in enzyme technology lowering the cost to about \$0.10–0.20/gal (American Institute of Chemical Engineering 2004; American Chemical Society 2005), and such a price would bring the overall cost of ethanol in our simple analysis to something under \$2.00/gal. Yet, this price would require protein costs of about \$1.00/lb or less, but no offers are known to sell enzyme at such a price. Thus, it appears that enzymes are still very expensive, with costs of about \$1.50/gal possible, and such high costs would prevent cellulosic ethanol from being competitive when using conventional enzymes.

Overall, this analysis shows that low-cost ethanol is possible from cellulosic biomass if we can bring enzyme costs down, and a particularly promising route to this end is through development of organisms that can both make enzymes and ferment the sugars released, as outlined at the beginning of this chapter. In addition to reducing process steps and capital and operating costs associated with separate enzyme production, the most important advantage to this approach called CBP is the anaerobic production of the enzymes needed to break down cellulose and hemicellulose to sugars. This feature overcomes a major barrier to current aerobic enzyme production methods that use huge amounts of energy to compress air and agitate the enzyme production vessels intensely to promote sufficient respiration by the organisms to grow and produce enzymes. Furthermore, such high-energy inputs also translate into major power drains on the facility and the need to provide very expensive cooling to remove all the heat generated by such intense aeration and agitation. Thus, unless enzymes are developed with much higher specific activities than historically seen, the best course to low costs appears to be to follow a CBP strategy.

In closing this section, it is vital to point out that the economics of ethanol production are very site specific and for that reason hard to generalize. Such aspects as labor rates and skill levels, biomass type and availability, competing demands for feedstocks, access to raw materials, transport of raw materials and finished products to and from the site, and access to markets can have a large effect on costs. In addition, while this rough analysis has been applied to corn stover because of its relatively large production, other agricultural residues may be more attractive due to cost, susceptibility to conversion, coproduct opportunities, and yields. Thus, one size does not fit all, and careful consideration should be given to regional factors before applying rigid economic evaluations.

Closing Thoughts

Agricultural residues can provide an attractive feedstock for cellulosic ethanol production in the near term because of their current availability, and many with high cellulose and hemicellulose content are amenable to conversion to ethanol with high yields. However, in estimating potential contribution to large-scale fuel production, consideration must be given to how much can be removed without problematic environmental consequences such as depletion of soil carbon and soil erosion. In addition, the cost of gathering and transport must be factored in, and collection strategies should be employed that minimize collection of dirt and stones. Storage techniques must also be developed that are low in cost but result in little degradation of the feedstock. And we must be sure that the sugars can be extracted from the carbohydrate fractions with high yield.

Because of the large impact feedstocks have on overall costs, selection of low-cost residues can be particularly important for overcoming the many obstacles to implementation of cellulosic ethanol technology for the first time in the near term including perceived risk and the associated high rates of return on capital, overdesign to compensate for risk, suboptimal facility sizes that keep investment costs lower but fail to capitalize on economies of scale, and other disadvantageous burdens. For example, cutting the cost by \$30/dry ton can reduce cash costs by over \$0.35/gal of ethanol produced. However, even then taking advantage of other economic levers such as integration into an existing fermentation or power facility to reduce capital costs; production of valuable coproducts from lignin, minerals, or other components; and use of low-cost debt financing through partnerships with municipalities or others can have a tremendous impact on commercial success (Wyman and Goodman 1993). Unfortunately, given the great fluctuations in petroleum prices, all of these factors may not be enough to overcome the huge obstacles facing first-time implementation, and government policy would make a major impact on bringing the technology into play before dire economic situations make it sufficiently profitable for the first projects to be successful (Wyman 2007). Although such assistance could take many forms, it must be structured in a manner that will not strand huge investments by the private sector while still ensuring rigorous due diligence that will result in economically viable projects. For example, government investments as an equity partner would buy down the high capital costs of first projects, show the private sector of the government's seriousness, and provide a payback to the government. Once in place, significant learning curve improvements and technology advances will lead to lower costs that can compete without government support. However, because huge lead times are needed to build up meaningful capacity, such a commitment must be made sooner rather than later if we really hope to reduce our mounting dependence on oil imports and have any hope of affecting buildup of CO₂ and other GHGs. Otherwise, we will continue to twiddle our thumbs while glaciers melt and coral reefs die.

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Chapter 10

Hydrothermal Liquefaction to Convert Biomass into Crude Oil

Yuanhui Zhang

Abstract

All fossil fuels found in nature—petroleum, natural gas, and coal, based on biogenic hypothesis—are formed through processes of thermochemical conversion (TCC) from biomass buried beneath the ground and subjected to millions of years of high temperature and pressure. In particular, existing theories attribute that petroleum is from diatoms (algae) and deceased creatures and coal is from deposited plants.

TCC is a chemical reforming process of biomass in a heated and usually pressurized, oxygen deprived enclosure, where long-chain organic compounds (solid biomass) break into short-chain hydrocarbons such as syngas or oil. TCC is a broad term that includes gasification, including the Fisher-Tropsch process, direct liquefaction, hydrothermal liquefaction, and pyrolysis. Gasification of biomass produces a mixture of hydrogen and carbon monoxide, commonly called syngas. The syngas is then reformed into liquid oil with the presence of a catalyst. Pyrolysis is a heating process of dried biomass to directly produce syngas and oil. Both gasification and pyrolysis require dried biomass as feedstock, and the processes occur in an environment higher than 600°C. The hydrothermal liquefaction (HTL) involves direct liquefaction of biomass, with the presence of water and perhaps some catalysts, to directly convert biomass into liquid oil, with a reacting temperature of lower than 400°C.

This chapter only covers the topic of HTL of biomass. Biomass feedstocks include biowaste (manure and food processing waste), lignocellulose (crop residue), and algae. The chapter is in two parts. The first part covers HTL fundamentals based on the current knowledge, and the second part is a summary of state-of-the-art knowledge of HTL for various feedstocks. The author has attempted to organize this chapter for a variety of readers who are interested in the topic of HTL, including students and professionals.

Fundamentals of HTL

HTL, also called hydrous pyrolysis, is a process for the reduction of complex organic materials such as bio-waste or biomass into crude oil and other chemicals. It mimics the natural geological processes thought to be involved in the production of fossil fuels. HTL is one of the processes of a general term of TCC which includes gasification, liquefaction, HTL, and pyrolysis. There is a general consensus that all fossil fuels found in nature—petroleum, natural gas, and coal, based on biogenic hypothesis—are formed through processes of TCC from biomass buried beneath the ground and subjected to millions of years of high temperature and pressure. In particular, existing theories attribute that petroleum is from diatoms (algae) and deceased creatures and coal is from deposited plants. Gasification of biomass produces a mixture of hydrogen and carbon monoxide, commonly called syngas. The syngas is then reformed into liquid oil with the presence of a catalyst. Pyrolysis is a heating process of dried biomass to directly produce syngas and oil. Both gasification and pyrolysis require dried biomass as feedstock, and the processes occur in an environment higher than 600°C. HTL involves direct liquefaction of biomass, with the presence of water and perhaps some catalysts, to directly convert biomass into liquid oil, with a reacting temperature of less than 400°C.

HTL has different pathways for the biomass feedstock. Unlike biological treatment such as anaerobic digestion, HTL converts feedstock into oil rather than gases or alcohol. There are some unique features of the HTL process and its product compared with other biological processes. First, the end product is crude oil which has a much higher energy content than syngas or alcohol. And second, if the feedstock contains a lot of water, HTL does not require drying as gasification or pyrolysis. The drying process typically takes large quantities of energy and time. The energy used to heat up the feedstock in the HTL process could be recovered effectively with the existing technology.

HTL may have two pathways from biomass to biofuel: (1) direct conversion of biomass or (2) pretreatment of biomass and then fermentation. For the biomass with little lignocellulosic fraction—such as waste streams from animal, human, and food processing—it can be directly converted into biofuel thermochemically. Pretreatment is currently a bottleneck in the conversion of cellulosic feedstock. HTL may hold a substantially greater potential to shorten the fermentation time of lignocellulose. Traditionally, acid hydrolysis was commonly used to convert lignocellulosic materials to monosaccharides, but the high concentration of acids used in hydrolysis requires extensive waste treatment or recovery costs.

The Role of Water in HTL

Water plays an essential role in HTL. It is therefore critical to understand the fundamentals of water chemistry when subjected to high temperature conditions. Water is rather benign and will not likely react with organic molecules under standard environmental conditions (20°C and 101,325 kPa). However, when the temperature increases, two properties of water molecules change substantially. First, the relative permittivity (dielectric constant), ϵ_r , of water decreases quickly when the temperature increases. When the thermal energy increases, the shared electron by oxygen and hydrogen atoms tends to circulate more evenly and the electronegativity of the oxygen molecule is reduced (less polar). For example, when temperature increases from 25°C to 300°C, the relative permittivity decreases from 78.85 to 19.66, resulting in water molecules from very polar to fairly nonpolar, in a relative term. This polarity

change makes water more affinitive to the organic hydrocarbons, most of which are nonpolar molecules.

Second, the dissociation of water dramatically increases with the increase of temperature. Water, like any other aqueous solutions, split into H^+ and OH^- ions in hydrolysis or dissociation. This process is reversible and the rate is sufficiently rapid so it can be considered to be in equilibrium at any instant. Based on Arrhenius reaction rate, the equilibrium constant (or the dissociation constant), K_w , affected by the temperature change, can be written as (Benjamin 2002):

$$\frac{K_{w1}}{K_{w2}} = \exp \left[\frac{\Delta E_{Ar}}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right) \right] \quad (1)$$

where K_{w1} and K_{w2} are equilibrium constant at temperatures T_1 and T_2 , respectively; ΔE_{Ar} is the net change in heat content of the molecules in the overall reaction, also called the molar enthalpy of reaction; R is the universal gas constant, and T is the absolute temperature in Kelvin. ΔE_{Ar} is an empirical constant specific to particular reaction and in units of energy per mole.

The effect of temperature on water dissociation is illustrated in Figure 10.1, where the left side vertical axis is in $pK_w = -\log_{10}(K_w)$, and the right side vertical axis is the ratio of K_w to the K_{w0} . K_{w0} is the water dissociation constant at a temperature of 25°C at atmospheric pressure, and is 10^{-14} . From Figure 10.1, water molecules dissociation constant at 300°C is about 500 times higher than that of 25°C at atmospheric pressure. The increase in the dissociation constant will increase the rate of both acid- and base-catalyzed reactions in water far beyond the natural acceleration due to increased temperature.

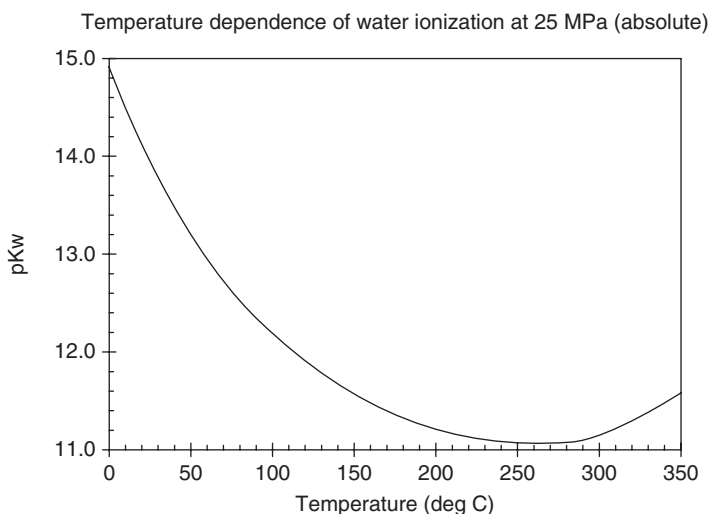


Figure 10.1. Effect of temperature on water dissociation constant at 25 MPa. The dissociation constant K_w is expressed as pK_w , where $pK_w = -\log_{10}(K_w)$. (IAPWS 2004).

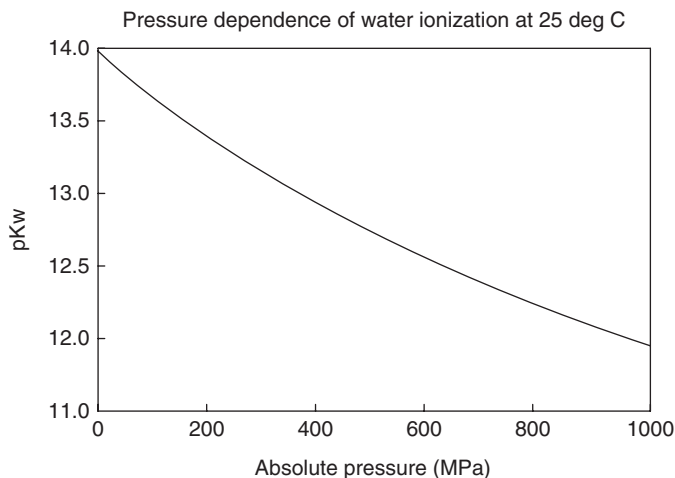


Figure 10.2. Effect of pressure temperature on water dissociation constant K_w at temperature of 25°C. The dissociation constant K_w is expressed as pK_w , where $pK_w = -\log_{10}(K_w)$.

There is also a (usually small) dependence on pressure (ionization increases with increasing pressure). The dependence of the water ionization on temperature and pressure has been fully investigated (Figure 10.2) and a standard formulation exists (IAPWS 2004).

For the above two reasons, water at high temperatures becomes a good solvent for hydrocarbons that are typically nonpolar hydrophobic under standard environmental conditions. These changes in physical properties make the solvent properties of water at 300°C roughly equivalent to those of acetone at 25°C. Ionic reactions of organics should be favored by increased solubility in water. The enhancement of this solubility of hydrocarbons in water will further enhance the possibilities of contact of dissociated H^+ with hydrocarbons, hence accelerate the activities of hydrolysis.

The dramatic changes in the physical and chemical properties of water as temperature increases suggest the possibility of organic chemical reactions to take place (Siskin and Katritzky 1991; Kruse and Gawlik 2003). In addition, water has the ability to carry out condensation, cleavage, and hydrolysis reactions, and to affect selective ionic chemistry, which is not accessible thermally, largely due to changes in its chemical and physical properties, which become more compatible with the reactions of organics as the temperature is increased.

Some classes of organic molecules in biomass proved very susceptible to water's influence. Hot water as a reactant and catalyst likely creates a second pathway for the cascade of molecular transformations that leads to oil. In this pathway, water causes organic material to disintegrate and reform (by adding H^+ to open carbon bond) into fragments that then transform into hydrocarbons. This implies that hot water becomes a catalyst for a series of ionic reactions. The acidic and basic nature of hot water—rather than heat—drives this cascade. For example, water may function first as a base, nibbling away at certain linkages in the organic material. As new molecular fragments build up and modify the reaction environment, water can change its catalytic nature. It can then act as an acid, accelerating different reactions. The resulting products attack parts of the remaining molecules, further speeding the breakdown (Siskin and Katritzky 1991). The above analysis may also help to explain why HTL will more likely directly convert biomass into oil than pyrolysis in which water is not involved.

Feedstocks and Their Primary Compounds

Agricultural biomass and biowaste include crop residues and wood, food processing waste, animal manure, and algae. Crop residues and wood primarily contain lignocellulose, while animal and food processing waste contains lipids, protein, and usually small amounts of lignocellulose (except ruminant animal manure). In this section, the primary compounds and structure in these feedstocks will be summarized and the potential functions in hydrothermal are explored, in the hope that it provides some bases to understand the interaction of carbohydrates with water (H^+ or H^* radicals).

Lignocellulose

Lignocellulosic compounds belong to the carbohydrates group of organic compounds. Carbohydrates are hydroxy aldehydes, hydroxy ketones, or substances derived from them. It is the principal substance that composes plants. The carbohydrates in swine manure come from both digested and undigested feed, and food processing waste contains carbohydrates food and lignocellulosic sources.

Glucose is one of the simplest monosaccharides. The isomers of glucose are shown in Figure 10.3. There are four chiral carbon atoms in the molecule. The carbon atoms at the end of the molecule do not hold four different groups and are not chiral. D-glucose forms a cyclic molecule by an addition reaction involving the carbonyl group and a hydroxyl group. The ring formation produces a new chiral center, and two isomers of D-glucose exist that differ in the orientation of the new OH group (Figure 10.4). Notice the α form of the OH group of the extreme right right-hand carbon atom is on the same side of the ring as the OH group of the adjacent carbon atom. In aqueous solution, α and β forms of the D-glucose exist in equilibrium, together with a low concentration of the open-chain form.

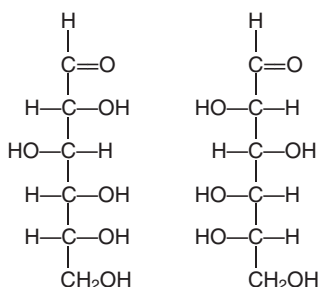


Figure 10.3. Optical isomers of glucose: left—D-glucose and right—L-glucose.

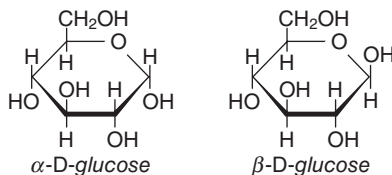


Figure 10.4. Ring forms of glucose.

Cellulose is a polysaccharide that only yields D-glucose upon hydrolysis. The number of D-glucose units in the molecular structures is estimated to be as high as several thousands. The D-glucose units of cellulose are linked in long chains in β combination shown in Figure 10.5. The structure is stabilized by hydrogen bonds between adjacent D-glucose units in the same strand. Cellulose occurs in fibrils brought about by hydrogen bonds among different strands.

Hemicelluloses are polysaccharides that are chemically related to cellulose, having backbones of 1,4- β -linked major sugar units, and being morphologically strongly associated with cellulose in the plant cell walls as well as to lignin in lignified cell walls. These polysaccharides are generally heterogeneous, built up of different hexoses (C6-sugars) and pentoses (C5-sugars), sometimes in addition to uronic acids. They have a lower degree of polymerization than cellulose (100–200 units), are largely soluble in alkali, and also more easily hydrolyzed (Figure 10.6).

The chemical structure of lignin is more complex than cellulose and hemicellulose. It resembles a network of aromatic compounds linked together in a more random fashion. The structure varies depending on source. To illustrate, the structure of a possible lignin molecule is shown in Figure 10.7. Lignin has high carbon content typically more than 60% and about 30% oxygen. Although in smaller amounts than cellulose, lignin represents about half of the available combustible energy in naturally occurring sources (Glasser 1985). Thermal decomposition of lignin occurs above 280°C depending on the source of lignin (Chornet and Overend 1985).

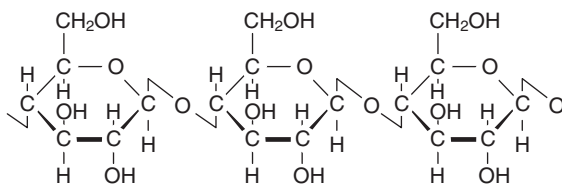


Figure 10.5. Chemical structure of cellulose.

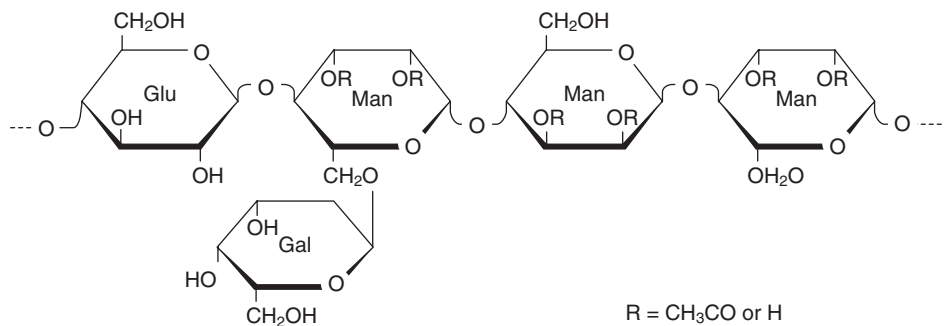


Figure 10.6. Chemical structure of hemicellulose.

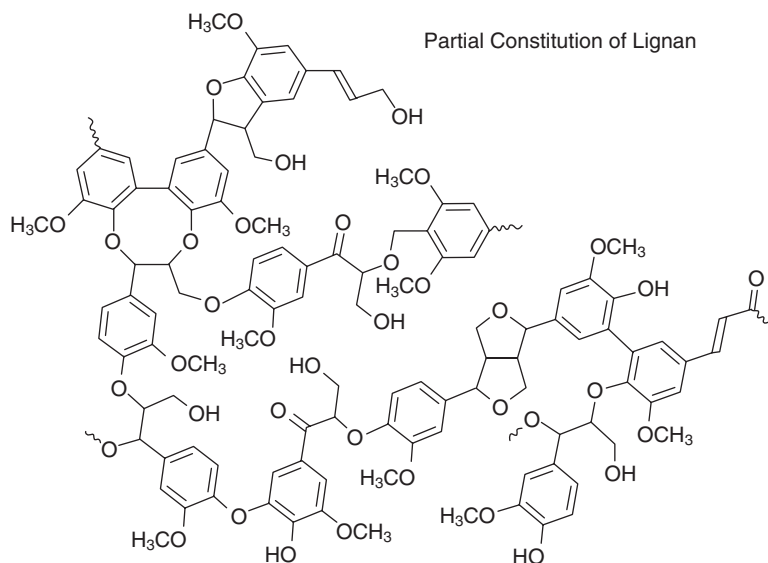


Figure 10.7. An example of chemical structure of lignin.

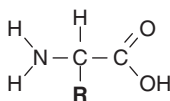


Figure 10.8. General structure of amino acid compounds.

Proteins and Amino Acids

Proteins are macromolecules formed from simpler compounds, α -amino acids. An α -amino acid is a carboxylic acid that has an amino group bonded to the carbon atom next to the carboxyl group. The designation α denotes the position of the amino group. The carbon atom adjacent to the carboxyl group is called the α -carbon atom. The general formula for an amino acid compound is shown in Figure 10.8. In the protein structure, amino acids are linked together by peptide bonds forming the long chains. These bonds are easily broken at high temperatures resulting in the formation of amino acids. Animal manure and food processing waste are rich in protein contents. For example, proteins comprise about 25% of the total solids in swine manure.

The radical, R, in the formula shown in Figure 10.8 differentiates the types of amino acids. The R may be simple hydrocarbons, ring compounds, additional amino or carboxylic groups, or -SH or hydroxyl groups. Alanine, leucine, aspartic acid (aspartate), and glutamic acid (glutamate) account for half of the total amino acids in swine manure (Figure 10.9). Proteins and amino acids are the major sources of organic nitrogen present in swine manure. Organic sulfur is mainly from two particular amino acids, cysteine (R = -CH₂-SH) and methionine (R = -CH₂CH₂-S-CH₃).

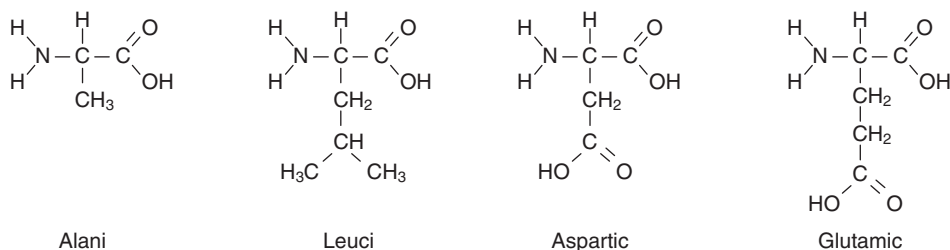


Figure 10.9. Structures of dominant amino acids in swine manure.

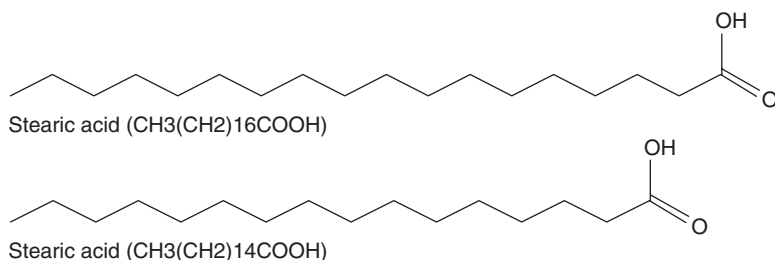


Figure 10.10. Structures of dominant fatty acids in swine manure.

Lipids

Lipids are substances that can be dissolved away from biological material by solvents that are nonpolar or slightly polar. Since the classification is based on solubility, not structure, a wide variety of compounds fall under lipids. Fatty acids are long straight-chain carboxylic acids some of which are saturated and some of which contain one or more double bonds. Almost all fatty acids isolated from natural sources contain an even number of carbon atoms. Among the fatty acids identified in swine manure, stearic acid and palmitic acid are most dominant (Figure 10.10).

Possible Hydrothermal Pathways

HTL is similar to the geological processes that produced the fossil fuels used today, except that the technological process occurs in a time frame measured in minutes instead of geological time. HTL is a chemical reforming process of biomass in a heated and usually pressurized, oxygen deprived enclosure, where long-chain organic compounds (solid biomass) break into short-chain hydrocarbons. All fossil fuels found in nature—petroleum, natural gas, and coal, based on biogenic hypothesis—are formed through HTLs from biomass buried beneath the ground and subjected to high temperature and pressure. Simple heating without water (pyrolysis or anhydrous pyrolysis) has long been considered to take place naturally during the catagenesis of kerogens to form fossil fuels. In recent decades, it has been found that water under pressure causes more efficient breakdown of kerogens at lower temperatures than without it (Siskin and Katritzky 1991; Pennisi 1993). The carbon isotope ratio of natural gas also suggests that hydrogen from water has been added during creation of the gas, in addition to the formation of oil. The state of fossil fuels (solids, liquid, or gaseous) depends

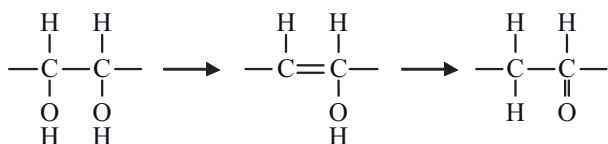
on the composition of feedstocks and environmental conditions, including temperature, pressure, retention time, and presence of particular catalysts.

The exact pathways of HTL to produce crude oil from biomass remain unclear, and additional research is needed. The following examples may give some hints of possible pathways of HTL of bio-waste feedstock. In a study by Appell et al. (1975), one of the mechanisms for the conversion of carbohydrates into oil that was consistent with the results they obtained was as follows:

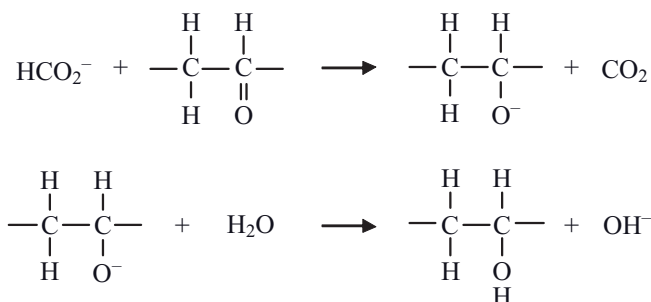
Sodium carbonate reacts with carbon monoxide and water to yield sodium formate:



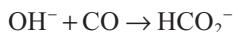
Vicinal hydroxy groups in the carbohydrates undergo dehydration to form an enol followed by isomerization to a ketone:



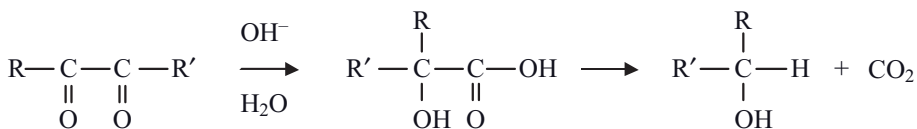
The newly formed carbonyl group is reduced to the corresponding alcohol with formate ion and water:



The hydroxyl ion then reacts with additional carbon monoxide to regenerate the formate ion.



A variety of side reactions may occur and the final product is a complex mixture of compounds. One of the beneficial side reactions occurs in alkaline conditions. Carbonyl groups tend to migrate along the carbon backbone. When two carbonyl groups become vicinal, a benzylic type of rearrangement occurs, yielding a hydroxy acid. The hydroxy acid readily decarboxylates causing a net effect of reducing the remainder of the carbohydrate derived molecule.



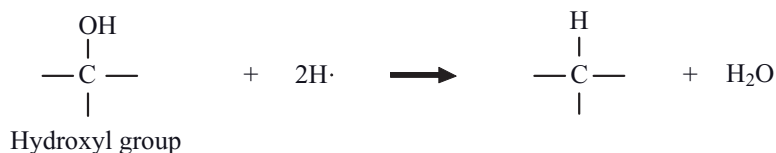
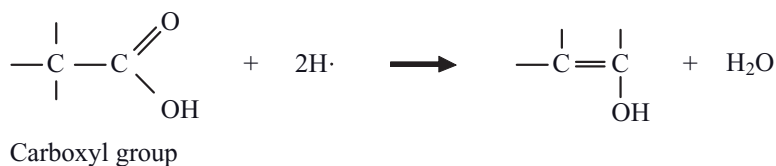
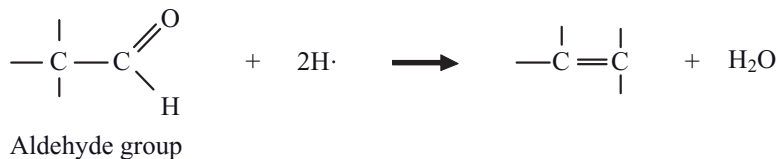
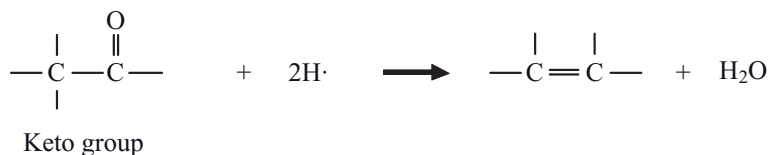
This type of reaction is beneficial to HTL because it leads to the formation of paraffin-type structures, which has less oxygen than the original compounds. In addition, the reaction happens by disproportionation and does not require any additional reducing agent.

Aldol condensation may also be part of the reaction process. Aldol condensation occurs between a carbonyl group on one molecule and two hydrogens on another molecule with the elimination of water. The condensation product is a high-molecular weight compound typically with high viscosity. Condensation reactions become a major pathway in the absence of reducing agents such as carbon monoxide and hydrogen. Reducing agents keep the carbonyl content of the reactant system sufficiently low so that liquid instead of solid products are formed.

In a study by Appell et al. (1980), the authors believed that the free hydrogen radical ($\text{H}\cdot$), not the hydrogen molecule (H_2), participates in the chemical conversion reactions. Thus, they concluded that the addition of carbon monoxide (CO) to the process was more efficient than the addition of hydrogen gas. Based on the water-gas shift reaction, carbon monoxide reacts with water to form carbon dioxide and two hydrogen radicals.



In the presence of the hydrogen radicals, the oxygen is removed from the compounds containing carbonyl and hydroxyl groups, then form paraffin and water. A possible pathway is described in the following four reactions (He 2000).



The complexity of the chemical reactions involved in HTL can be attributed to the complex composition of feedstocks. According to Chornet and Overend (1985) and Vasilakos

and Austgen (1985), some of the reactions that may be involved in the liquefaction of carbonaceous materials are cracking and reduction of polymers such as lignin and lipids, hydrolysis of cellulose and hemicellulose to glucose and other simple sugars, hydrogenolysis in the presence of hydrogen, reduction of amino acids, reformation reactions via dehydration, decarboxylation, C-O and C-C bond ruptures, and hydrogenation of functional groups.

State of Knowledge of HTL of Biomass

HTL of Biowaste Streams

In this section, two topics are summarized: one is on HTL of different kinds of waste streams, including manure, sewage sludge, urban waste, and agricultural wastes. The other is on the mechanisms study of HTL.

Appell et al. (1970) are among those who first started the study on the HTL process of waste streams. Urban refuse, cellulosic wastes, and sewage sludge were tried as feedstocks in a 500-mL autoclave. Effects of two kinds of initial gases, CO and H₂, were compared. In the research, using CO as initial gas led to higher oil yield in the process. When using municipal refuse as feedstock, at 20 minutes, 380°C, and 1500 psi, the oil yield is 41% versus 18% for CO versus H₂. At 500 psi CO, 1 hour, 250°C, 20% NaHCO₃, oil yields of 37% and 35.5% were obtained from newsprint, pine needles, and twigs, respectively. For sewage sludge, without a catalyst, oil yield was 24.5%. Infrared and mass spectrometric analysis indicated the oils to be paraffinic and cycloparaffinic with the presence of carbonyl and carboxyl groups. High temperature product has a very small amount of aromatic material, probably phenolic, but the oil product at low temperature does not appear to have any aromatic compounds.

With cellulose as feedstock, at 2 hours, 350°C, 1500 psi initial pressure, a much higher benzene-soluble oil yield was obtained when using CO rather than using H₂ as initial process gas, 40% versus 5% solubility, respectively. CO as a process gas also resulted in a lower oxygen content in the oil (8.9% vs. 15.5%). Residue and oil have similar elemental composition but different structures, making them appear differently. Water-soluble fraction resulting from hydrolysis of the cellulose is believed to be a precursor of the oil because it can be converted to oil by recycling the aqueous solution through the process with subsequent charges of refuse.

Fu et al. (1974) conducted the HTL process on bovine manure. Bovine manure was hydrogenated and liquefied with the existence of hydrogen or synthesis gas (H₂:CO = 1:1) at temperatures of 330–425°C and operating pressures of 1500–3000 psi, in the presence of a recycled manure oil (or an alkyl naphthalene oil) and a Co-Mo catalyst. The Co-Mo catalyst could be a good choice for increasing oil yield. At 380°C/425°C, 3000 psi, better oil yield was observed when the Co-Mo catalyst was present. Of all the variables investigated, temperature has the most dramatic effect on properties of the oil product. Although there were no significant improvements for conversion or oil yield, significant improvements in oil product quality were observed, with increased carbon content, decreased oxygen content, and reduced viscosity. High-resolution mass spectrometry analysis of oil produced at 380°C indicates that the main components are alicyclic hydrocarbons, N-containing heterocyclic compounds, and alkyl phenols with carbon numbers ranging from C8-C18. All carbon dioxide was produced before the reaction mixture reached 380°C (before zero time).

Minowa et al. (1995a) applied the HTL process to artificial garbage prepared by mixing cabbage, boiled rice, boiled and dried sardines, butter, and the shell of short-necked clams. Three temperatures (250, 300, and 340°C) and three retention times (0.1, 0.5, and 2 hours) were tested without a catalyst. Oil yield and its properties strongly depended on the catalyst addition and reaction temperature, while retention time showed no significant effect. Highest oil yield of 27.6% on an organic basis was obtained at 340°C, 18 MPa pressure and 0.5 h retention time with a catalyst. The oil had a heating value of 36 MJ/kg and a viscosity of 53,000 mPa·s at 50°C.

Suzuki et al. (1988) investigated optimum starting materials and catalyst loading for conversion of sewage sludge to heavy oil. Various kinds of sewage sludge were liquefied at 300°C, 12 MPa, and a catalyst loading of 0–20 wt %. Of digested sludge, raw waste activated sludge, raw primary sludge, and raw mixed sludge, have higher oil yields averaging 43%. The nature of the sludge had no significant influence on the elemental composition and heating value of oils obtained. Catalyst loading had no significant influence on the properties of oil products. Results showed a nearly linear relationship between crude fat content and the amount of oil fraction in the starting materials. Calcium salts could possibly have some catalytic effect on the liquefaction reactions.

Murakami et al. (1990) converted activated sludge from a cornstarch processing plant into oil with the HTL process. A 100 cm³ autoclave and N₂ initial gas were used, and the working pressure was maintained at the saturated vapor pressure of water at the required temperature plus 3.0 MPa. Effects of temperature, Na₂CO₃ catalyst loading, and holding time were studied. Results showed that maximum oil yield was 30% at 300°C, 60-minute retention time without a catalyst. Oil yield was not significantly affected by catalyst loading. Properties of the oil product were not influenced to any great extent by temperatures between 225–300°C, while the aqueous phase product yield and solid residue decreased as temperature rose. Heavy oil production at temperature as low as 250°C is possible, provided the reaction is carried out with sufficient retention time. According to their energy balance, the liquefaction could be a self-sufficient process under certain conditions.

A demonstration plant with a capacity for processing up to 5 t/d as dewatered sludge was operated at 300°C, 10 MPa, (feedstock, moisture content ~80%, VS ~80%; Itoh et al. 1994). The sludge had been dewatered by belt press dehydrator after adding a polyelectrolyte coagulant. As a result, 48% mass of the organic materials in the sludge were converted into heavy oil, and a quarter of the oil was separated from the reaction mixture by high pressure distillation with a distillate ratio of 0.33. Heating value of the heavy oils distilled were 37–39 MJ/kg, while that left in the bottom was 31–35 MJ/kg. Energy balance was calculated based on the pilot plant data collected. For a plant of 60 t/d dewatered sludge, the sludge is treated without any auxiliary fuel, and 1.5 tons of heavy oil is produced as surplus energy. In conclusion, the treatment of sewage sludge by this method could be sufficiently profitable.

He et al. (2000a,b; 2001a,b,c) studied HTL for swine manure. The process was evaluated by oil production efficiency and waste reduction efficiency. The oil product was analyzed for its benzene solubility, elemental composition, and heating values. Thermogravimetric properties and viscosity were also measured on selected oil samples. The difference of chemical oxygen demands before and after the HTL process was used as the waste reduction efficiency. The key factors of the HTL process were the operating temperature, the retention time, and the addition of a process gas. The operating temperature effect was studied in the range of 275–350°C. The suggested operating temperatures are between 295°C and 305°C. A process gas addition was necessary to achieve an oil product. Without the process gas, no oil products formed. The process gases investigated include carbon monoxide, hydrogen, nitrogen, carbon

dioxide, and compressed air. Carbon monoxide was the most effective gas for the process. Retention time is another important factor. The necessary retention time to achieve an oil product was largely related to the operating temperatures. When the operating temperatures were 295°C–305°C, the retention time was 15–30 minutes. Based on an average of 135 different oil samples, 62 wt % of the volatile solids were converted to oil. The waste strength was reduced by 60% to 70%. The highest oil production efficiency was 80 wt %. The average carbon and hydrogen contents were as high as 72 wt % and 9 wt %, respectively. The heating values for 80% of the oil products ranged from 32,000 to 36,700 kJ/kg. The results showed that HTL of swine manure to produce oil is technically feasible, and could be a promising technology for waste reduction and renewable energy production.

Since a continuous system is more applicable for scale-up operations, a small-scale continuous HTL (CHTL) reactor system was developed (Ocfemia et al., 2006a) to aid in the evaluation of the technical feasibility and economical viability of a pilot plant that is capable of producing oil from swine manure. The effects of operating conditions, including temperature, pressure, hydraulic residence time, and use of process gas, were evaluated in order to determine the optimal process condition. The composition of the different product streams (i.e., oil, aqueous, and gas) was determined to better understand the mechanics of the reaction process and to provide information for further developments. The CHTL reactor system was composed of a high-pressure slurry feeder, a process gas feeder, a continuous-stirred tank reactor, a products separation vessel, and process controllers. It had a capacity to process up to 48 kg of manure slurry per day. The operating parameters—temperature, pressure, residence time, and the use of CO—were all found to affect oil yield (Ocfemia et al., 2006b). The interaction between operating temperature and pressure was evident. The highest yield of 70% (based on volatile solids content of the manure feedstock) was found to be in the region where temperature was about 300°C and pressure was 10 MPa. Yield was found to increase with hydraulic residence time, but there was a diminishing benefit after 60 minutes. The addition of CO in the process did not improve the oil yield, but produced a more fluid oil product. The heating value of the oil product ranged from 25,176 kJ/kg to 33,065 kJ/kg with the highest value at the operating condition of 305°C, 10.3 MPa, and 80 minutes hydraulic residence time. The energy balance based on oil heating value and energy used for heating the feedstock material to the operating temperature showed that the process was a net energy producer.

Elemental analysis of the crude oil showed that the average (from all tests) carbon and hydrogen content of the oil was $62.7\% \pm 6.4\%$ and $9.6\% \pm 0.4\%$, respectively. The nitrogen content was high with a value of $3.9\% \pm 0.3\%$. The sulfur content of the oil was low with a value of $0.3\% \pm 0.1\%$. The composition of the oil based on SARA analysis showed that the oil was primarily resins (~45%) and asphaltenes (~44%) with small amounts of saturates (~3%) and aromatics (~2%). The boiling point distribution of the oil showed that the majority of the compounds were in the boiling point range of 316–482°C.

In Ocfemia et al.'s study (2006b), the aqueous product was found to contain volatile organic compounds, primarily ketones and benzenyl compounds. Of dominance was acetone, which accounted for 0.7 mg/L of the aqueous product. The total N content was 6.1 ± 1.9 g/L. Phosphorus as phosphate was 1.0 ± 0.3 g/L. The total aqueous K was 1.5 ± 0.8 g/L. The main gas product was CO₂ accounting for ~98% of the total. Carbon monoxide accounted for about ~2%. Hydrocarbons, including methane and ethane, were found to have a combined concentration of 299 ppmv. Very small amounts of aromatic compounds, including benzene, ethylbenzene, toluene, and styrene, were also detected.

Dote et al. (1992) studied the oil samples separated by steam distillation after liquefaction of sewage sludge. Oil was fractionated to strongly acidic, weakly acidic, basic, and neutral fractions by acid–base extraction. The total recovery of fractions was 77%. Each fraction was analyzed by gas chromatography and mass-spectrometry (GC-MS), and 71 types of compounds were identified with reasonable certainty. No strongly acidic fraction was obtained. The weak acidic fraction, comprising 2% of the oil, was exclusively composed of phenolic compounds. The basic fraction, comprising 20% of the oil, was exclusively composed of pyridines, pyrazines, quinolines, amines, and methylphenylacetamide. The neutral fraction, comprising 56% of the oil, was exclusively composed of aliphatic compounds, alicyclic compounds, alcohols, ketones, aromatic compounds, sulfur-containing compounds, nitrogen-containing compounds, and oxygen-containing heterocyclic compounds.

Dote et al. (1996) studied the distribution of nitrogen in the products for direct liquefaction of protein-contained biomass. Albumin from an egg was used as feedstock, and tests were run at 150–340°C, 0.5 hours, and 2 hours, w/o Na₂CO₃ as a catalyst. The maximum oil yield was 10%, much less than that for practical feedstocks (all above 30%). Nitrogen distributed to oil was 5% at most, much less than that for practical feedstocks (30%–45%). Other practical feedstocks contain other elements such as cellulose and lipid, which may increase the amount of oil converted from protein or react with nitrogen-containing compounds produced from protein during the conversion. No distribution of nitrogen to oil occurred below 150°C, and the distribution was completed by 250°C. The majority of the nitrogen in albumin (80%) was distributed to the aqueous phase, and albumin was decomposed to ammonia, not to amino acids. Sodium carbonate seemed to prevent the distribution of nitrogen to oil.

Minowa et al. (2004) used glucose and glycine as model compounds of carbohydrates and proteins, to study the mechanisms of hydrothermal reactions. There were 1.8 g glucose, 0.75 g glycine, and 30 mL distilled water charged into 100 mL autoclave. N₂ was used as initial gas and was added to 3 MPa pressure. Temperatures of 150–350°C were studied. It was concluded that at 150°C, the main reaction was Maillard reaction and melanoidin was formed. At 200°C, produced melanoidin was decomposed to form char, gas, and aqueous-soluble materials. Oil production started at 250°C, and oil yield increased with reaction temperature. It appears that oil is formed through the secondary decomposition of the decomposed products in the aqueous phase and not directly formed from melanoidin. Char was formed in the low reaction temperature range from 150°C to 200°C, and char yield was almost the same over 200°C. No pathway from oil to char is significant. Produced ammonia could inhibit the char formation from oil.

It appears that fatty acid and lipid are the main reactants of HTL. The predominant HTL reaction below 300°C is considered to be distillation of aliphatic compounds. The existence of considerable straight chain compounds (C13–C22) suggests that the aliphatic compound is the main resource of derived oil. A large quantity of nitrogenous compounds (mainly composed of amide and cyanide) in the oil suggests that protein is widely involved in the HTL reaction, possibly by peptide bond splitting and amino acid conversion dehydration. Within 300–450°C, the protein conversion reaction intensifies, and its principal structural bond (peptide bond) begins to react. Saccharide reaction mainly belongs to the splitting of branched chain and group transfer while considerable dehydration and cyclization of the main chain still appears not to be dominant. A simplified reaction model of HTL for sewage sludge consists of two serial competitive reactions—producing volatile matter and char, respectively. Estimated Arrhenius kinetic parameters of the reaction model based on Thermogravimetric testing results were introduced.

It is worth mentioning that Huber et al. (2006) conducted a comprehensive review on biofuel producing methods, including gasification, liquefaction, and pyrolysis. Some chemical reaction mechanisms, oil synthesis, and upgrading methods were also included in that review.

HTL of Lignocellulose

The presence of liquid water is essential in the HTL of lignocellulose feedstock, even more important than for other types of biomass (manure and algae, for example). Aside from its role as a vehicle and catalyst carrier for the feedstock, water also serves as a solvent and reactant. The use of water as the solvent for HTL presents several advantages over other solvents. Water is simple to use, is relatively low cost, and is environmentally benign.

Water is an excellent medium for the intermediate hydrolysis of cellulose and other high-molecular weight carbohydrates to water-soluble sugars. The primary reaction in the conversion to oil likely involves the formation of low-molecular weight, water-soluble compounds such as glucose. Hydrogenation of sugars at mild temperatures produces polyols (hexitols and xylitol), which undergo further transformation into mixtures of glycerol, ethylene glycol, and propylene glycol (Chornet and Overend 1985). In another account, monomers such as glucose are further reduced with the presence of reducing compounds (Houminer and Patai, 1969). The sugar is de-oxygenated producing high carbon-hydrogen compounds.

Most organic compounds do not react with water under normal conditions. However, at temperatures between 250°C and 350°C, molecules in liquid water undergo chemical reactions. Previously, these reactions were only expected to occur in the presence of strong acid or base, but recent research indicates otherwise (Siskin and Katritzky 1991). Siskin and Katritzky (1991) have shown the geochemistry of the reactivity of organic molecules in hot water. Ester groups, which are bound in to the network of resource structures and serve as crosslinks, although thermally unreactive, are easily cleaved in water at 250–350°C (Siskin et al. 1990a). Similarly, benzyl aryl ethers were found to be more susceptible to cleavage under aqueous thermal conditions at 250°C. Cyclohexyl phenyl compounds with oxygen, sulfur, and nitrogen links are relatively unreactive thermally, but they readily cleave in water at 250°C to form methylcyclopentene together with phenol, thiophenol, or aniline, respectively (Siskin et al. 1990b). Benzonitriles, pyridinecarbonitriles, benzamides, and pyridinecarboxamides are almost unaffected by thermolysis, but are rapidly hydrolyzed in water at 250°C to the corresponding ammonium carboxylates (the nitriles via the amides). The ammonia formed autocatalyzes these hydrolysates and the subsequent decarboxylations (Katritzky et al. 1990). In the formation and depolymerization of resource materials, autocatalysis appears to be a major mechanistic pathway. During the diagenesis of kerogens, oxygen functionalities such as carboxylic acids, aldehydes, and alcohols are lost directly by cleavage and indirectly by condensation reactions that form methylene-bridged, ether, and ester cross-links. The cleavage reactions release water-soluble products such as carbon dioxide, formic acid, and formaldehyde.

HTL can convert lignocellulose into oil, but the yield is relatively low when no catalyst or solvent is used. Wang et al. (2008) compared the oil produced from different biomass—including legume straw, corn stalk, cotton stalk, and wheat straw—under hydrothermal conditions of temperature 350°C, residence time 2–3 hours, solid content 15% (dry mass), and pressure 10–13 MPa without using any catalyst. Hydrothermal experiments were carried out with 5.0 g biomass in a stainless tubular reactor which is 100 mm length by 10 mm internal diameter. The heating rate of reactor is about 10°C/min. The oil product was separated from

reaction mixture by distillation at 101–405°C and atmospheric pressure. Water and oil automatically separated into two phases in their study. Experimental results showed that oil yield is in the range of 5.2%–10.5% and both char and gas yield are more than 35% as the total biomass was almost completely converted. In addition to CO₂, gas products contained about 4.4%–8% H₂ and 5.5%–13.3% CO. Analysis of the oil product indicated that oil mainly consists of alkanes, cycloalkanes, and aromatic hydrocarbons. Based on these results, they concluded that the component of starting material had little effect on oil composition.

Tracing the origin of polyphenols, an abundant class of natural compounds, may contribute to awareness of reactions taking place upon high temperature treatment of waste materials. Luijkx et al. (1991) reported on the high yield of 1,2,4-benzenetriol from conversion of aqueous HMF as well as D-fructose. In a heated open tube reactor with 1.43 mm ID × 3.18 mm length, a 0.05 mole of HMF in water was converted 80%–90% with maximum 25% yield of 1,2,4-benzenetriol at about 330–350°C for 250 seconds. At 330°C for 185 seconds the 1,2,4-benzenetriol yield is about 9% from D-fructose. Those observations indicate that 1,2,4-benzenetriol is directly produced from HMF. Other identified compounds are less than 2%, including 4-oxopentanoic acid (levulinic acid), furaldehyde (furfural), and 1,4-benzenediol (hydroquinone).

To understand the formation mechanism of oil-like or tarry compounds, it is logical to start with the chemistry of HTL using model compounds which are present during the reaction. Luijkx et al. (1993) converted model compounds such as HMF derived from biomass to 1,2,4-benzenetriol with a yield of up to 46% at 50% HMF conversion at 290–400°C and 27.5 MPa. Experiments were conducted on a continuous process with a 4.8 mL tube reactor. It was found that 1,2,4-benzenetriol yield increased with residence time as HMF was converted at 290–380°C and that selectivity of 1,2,4-benzenetriol increased to 46% at 50% HMF conversion at 300–350°C followed by a decrease due to decomposition of 1,2,4-benzenetriol at high temperature and long residence time. In addition, distribution of products derived from HMF can be altered by pH changes, although 1,2,4-benzenetriol could be detected in the aqueous phase. It was concluded that HMF was a precursor of hydroxylated aromatics such as 1,2,4-benzenetriol in the HTL of biomass. However, a small amount of tar derived from HMF, which has a very high oxygen content (apparently 30%, and the usual oxygen content of hydrothermal oil products is about 18%) and was unstable due to polymerization. For that reason, HMF may not be considered as a representative compound for simulation of hydrothermal conversion of biomass.

Since the hydroxylated benzenes were identified in the aqueous product from hydrothermolysis of carbohydrates (Suortti 1983), the link between furan and hydroxylated benzenes was examined to further explore chemistry involved in HTL. Luijkx et al. (1994) studied the origin of hydroxylated benzenes from these furan derivatives from biomass under hydrothermal conditions. Hydrothermal conversion of furan derivatives was performed in a continuous tube reactor at 340°C, 27.5 MPa, residence time of 1–33 minutes, and feed concentration 0.01 M or 0.05 M with or without hydrochloric acid. Their results show that furan derivatives such as 2-acetylfuran, 2-propionylfuran, 5-methyl-2-furaldehyde can be directly converted to 1,2-benzenediol, 3-methyl, 1,2-benzenediol, and 1,4-benzenediol, respectively, without experience of smaller molecular fragments since only a few types of hydroxylated benzenes were obtained. Otherwise, a whole range of products would be collected. With catalysis of hydrochloric acid, acetic acid and propionic acids were produced as major products from conversion of furan due to cleavage of the furan ring. However, for 5-methyl-2-furaldehyde (which is found in hydrothermolysis as well as pyrolysis of carbon-hydrolysis of carbohydrates and HMF), 1,4-benzenediol was increased to about 30% due to

the presence of hydrochloric acid. For the conversion of HMF, it was found that it was unlikely to get 1,2,4-benzenetriol via electrocyclic mechanism. The formation of hydroxylated benzenes from furans such as HMF is probably via a hydrolytic furan ring opening followed by an intramolecular aldol condensation which is catalyzed by strong alkaline or sometimes by acid and subsequent dehydration.

With observation of a significant amount of CO_2 formed from HTL, it is interesting to know how CO_2 evolved from biomass under the hydrothermal condition. Luijkx et al. (1995) examined the role of deoxyhexonic acids in the hydrothermal decarboxylation of carbohydrates. Hydrothermal conversion was conducted at 340°C and 27.5 MPa in a continuous tubular reactor for residence times of 1–3 minutes. It was found that the temperature exerted an effect on reaction pathway of hydrothermolysis since almost no 3-deoxy-D-erythrohex-2-ulose was detected at temperatures less than 250°C with the presence of alkaline. Only small amounts of 3-deoxy-d-hexonic acid were observed in hydrothermolysis of a mixture of D-glucose and oligomers. Although 3- and 2-deoxyhexonic acid can decarboxylate, their contribution to CO_2 formation during hydrothermal conversion is limited when small amounts of deoxyhexonic acids formed during hydrothermal conversion of carbohydrates was considered. In addition, it seems that the addition of NaOH increases gas formation of CO but does not favor the formation of CO_2 .

The tar and char formation has been speculated by Chornet and Overend (1985). Chornet and Overend stated that the accessibility to cellulose chains was hindered due to surrounding the compounds. "In a pure pyrolytic context (i.e., carbonization), thermally induced rapid breakdown of the cellulosic chains results in intermediate compounds which are sterically hindered within the rigid lignin-rich structure of the compound, middle lamella, since lignin decomposes at higher temperatures than cellulose (Beall and Eickner 1970). The net effect is the random recombination of the intermediate compounds leading to tar and char formation" (Chornet and Overend 1985).

In the presence of a solvent, catalyst, and hydrogen, the oil yield from hydrothermal conversion of biomass could increase significantly. Kaufman et al. (1974) studied the conversion of cellulosic feed materials to liquid hydrocarbon fuels with newspaper as feedstock and nickel hydroxide as catalyst. The 20 wt % of powdered newspaper in mineral oil was processed in a 1-L continuous stirred tank reactor (CSTR) at $400\text{--}455^\circ\text{C}$, hydrogen pressure 34–102 atm with the presence of 0.2 wt % of $\text{Ni}(\text{OH})_2$ catalyst. Results show that decrease in temperature can lower both the carbon conversion and oil yield, while an increase of hydrogen pressure from 36.7 to 70.8 atm promotes oil yield from 5.2% to 46.1% at 453°C and 17.5 minutes space time and carbon conversion is constant (69.4% vs. 74.1%). The oil product would decompose to gas if exposed to $452\text{--}463^\circ\text{C}$ more than 20 minute space time.

Complete liquefaction and gasification of biomass can be achieved with a catalyst present in water and the degree of gasification changed with the catalyst to biomass ratios (Boocock et al. 1979). Boocock et al. (1980b) hydrothermally liquefied 150 g of air-dried poplar with particle size of a 0.5 mm mesh and 750 mL water in a 2-L magne drive packless autoclave under catalyst effect. Catalyst (20 g) causes the complete liquefaction and gasification of wood with 33.8% oil yield at 350°C , holding time 1–2 hours, and H_2 initial pressure 10.7 MPa. The catalyst obviously increased the consumption of H_2 and formation of CH_4 , but inhibited CO_2 , which implies that Raney Ni could catalyze formation of CH_4 from CO_2 and H_2 . With the decrease of activity of the Raney Ni catalyst, CO_2 selectivity increased while CH_4 formation decreased and biomass was completely converted. Results suggest that the catalyst was modified during the liquefaction process and that the spent catalyst did not promote the formation of methane, but enhanced the formation of carbon dioxide. It was thought that the oil

product was responsible for catalyst modification and found that reaction was not influenced by water to oil ratio. The spent (modified) catalyst retained its activity as there were no signs of solid residue in the autoclave. In addition, using a lower H_2 pressure slightly increases CO_2 formation. Without H_2 , the spent catalyst can also completely convert wood to viscous liquid at $350^\circ C$ and 2 hours, which is flowable at room temperature except for a much lower hydrogen content in the oil product. The oil product shows viscosities from 700–8000 mPa.s at room temperature, oxygen content about 10%–13%, specific gravity of 1.1, heating value about 35.3 MJ/kg, 97% benzene solubility, 55% diesel solubility, and 33% aromatic carbon content. The residence time was reduced to 30 minutes or less, and the catalyst was upgraded from Raney nickel to nickel from nickel salts.

Raney nickel catalyst can be substituted by a less exotic and hence less expensive, nickel catalyst. Boocock et al. (1982) investigated and reported the effectiveness of the Raney catalyst in detail. Wood (7-year-old hybrid poplar) was thermohydrolyzed in a 2-L packless magne drive autoclave with Raney catalyst at $340^\circ C$ for 2 hours. As temperature was more than $375^\circ C$, excessive char formation was noted. In a typical experiment 150 g dry wood and 20 g Raney catalyst were used with 750 mL water pressured under 1.7–8.3 MPa H_2 . The oil product tends to be more viscous, darker, and denser with the stabilization of a fresh catalyst taking place. Their results imply that fresh Raney Ni seems not able to catalyze the conversion of CO_2 and H_2 to CH_4 in the aqueous phase because the change in H_2 consumption is not significant. Therefore, CO_2 may be catalytically produced and CH_4 is probably formed directly by cracking processes in the presence of a catalyst. Raney Ni does oxidize and move to the aqueous phase, presumably to produce H_2 , but the amount is not large. Higher initial H_2 pressures favor hydrogen consumption and most of H_2 is consumed in methane formation. H_2 consumption increases with stirring rate (1300–1750 rpm) as initial pressure increases from 1.7 to 8.3 MPa (25–1200 psi). At 2300 rpm, oil viscosity can be lowered without a significant increase in H_2 usage. At the same time, H_2 uptake reduces the viscosity of oil and oil yield (separated by centrifuge and acetone) varies from 36.5% to 41%. The lower pH modified the Raney Ni and favored the CO_2 formation. In general C, H, and O content of the oil product were 73%, 8%, and 17.5%, respectively. Heating value of oil production was about 34 MJ/kg. The H/C is lightly lower in the oil than in the wood. Only 10% H appeared in methane and using H_2 is not theoretically required; its major function is to prevent the nickel from being oxidized and passing to the aqueous phase. With nickel carbonate as the catalyst, promising results of 80% C remained in the oil product and 55% wood H was retained in the oil. Nickel carbonate was reduced *in situ* to finely divided nickel, which presumably functioned as the catalyst (Boocock et al. 1980a). However, commercially available nickel powders did not appear to be as effective as Raney nickel or nickel produced *in situ* (Boocock et al. 1980a).

A recent study has also shown that a modified catalyst theoretically contributed to selectivity of catalyst that potentially makes conversion of biomass to biofuel more efficient in the presence of a catalyst (Teschner et al. 2008). Although heterogeneous catalytic conversion is a surface process, there is accumulating evidence, particularly from experiments applying *in situ* functional analysis, that the bulk and especially the subsurface region (the few layers below the surface) can play a key role in surface events. Reaction conditions (such as temperature and the ambient reactive gas) may not only reconstruct the top surface layer, but also may create added rows and valleys of atoms or even massively change the whole morphology of the catalytic particles (Teschner et al. 2008). Atoms that are part of the catalytic feed can dissolve in metallic particles, and can change the electronic structure of the surface, and dissolved species can even participate in the reaction; for example, alkyne hydrogenation on palladium.

Pd itself is usually even more active in hydrogenating the corresponding alkene to alkane (Teschner et al. 2008). The typical explanation (a thermodynamic view) is that the difference in the heat of adsorption of the feed alkyne and of the partial hydrogenation product alkene forces the intermediate product alkene to desorb and become replaced by the incoming alkyne of the feed (Teschner et al. 2008). A contrary example, ethylene could be adsorbed on a catalyst of Pd supported on silica while acetylene was present in the gas phase. This is possibly because the surface of catalysts is usually heterogeneous and can have discrete sites that facilitate selective adsorption (Teschner et al. 2008). Another fact is that carbonaceous deposits formed during reaction might substantially affect selectivity (Teschner et al. 2008). In addition, alkyne hydrogenation usually goes through an activation period, which strongly suggests that the catalyst is not identical to its “as-introduced” form. It was found that selectively hydrogenate 1-pentyne, the active state of Pd is a Pd-C surface phase (PdC), approximately three Pd layers thick (Teschner et al. 2008).

The *in situ* X-ray photoelectron spectroscopic measurement and *in situ* prompt gamma activation analysis (PGAA) were used to observe the hydrogenation process (Teschner et al. 2008). The amount of C incorporated within the top layers was 35–45 atomic % based on XPS investigation (Teschner et al. 2008). PGAA experiments show that the surface properties are necessarily decoupled from the bulk. The high concentration of dissolved carbon excludes H from populating the subsurface region and hence prevents total hydrogenation of alkynes. They are aware that many other factors, such as promoters in the form of a second metal or selective poison, can strongly modify the hydrogenation selectivity (Teschner et al. 2008). Their aim was to shed some light on the importance of subsurface chemistry in hydrogenation processes. They believed that a critical level of understanding of both surface and subsurface dynamics in these and other complex processes of heterogeneous catalysis is required. Although gas-phase alkynes hydrogenation on palladium catalysts is a surface process, they have shown that the population of the subsurface region by either C or H will determine the surface events.

Nickel catalyst has been shown to be helpful in stabilizing the liquid products and preventing charring. A relative cheaper catalyst, nickel carbonate, was examined. Beckman and Boocock (1983) used an 8-mL tubular reactor with adequate mixing and rapid heat-up rate to convert seven-year-old hybrid poplar in the presence of NiCO_3 . Results indicate that the oil yield was highest at a short residence time. At the same time, oxygen content of oil decreases from 25% to 17% during this period. The pH of the aqueous phase dropped due to carboxylic acids and phenolic compounds followed by an increase. The addition of nickel carbonate is deleterious to the liquefaction process under rapid heat-up in terms of C and H percentages in the wood that is retained oil. NiCO_3 and H_2 may not have a significant effect on oil yield, oil compositions, and pH of the aqueous phase. Degradation of wood begins at 230°C and 267°C because no oil was produced at 230°C. The results were interpreted that as slow heat-up proceeds, the wood is converted from a solid to a viscous tarry which is in turn liquefied in the presence of Ni or carbonized in the absence of Ni. At fast heat-up, wood is liquefied directly.

Besides nickel, other commercially available catalysts with different active sites such as Pd, Fe_2O_3 , NiO, and MoO_3 were used to convert lignin to oil in the presence of hydrogen. Meier et al. (1992) directly hydrogenated lignin in the aqueous phase over to a catalyst without using any solvents and pasting oil. In their experiments, 40 g of dry lignin with 3.5 g moisture inside mixed with 15 g of catalyst in a 250 mL autoclave under hydrogen pressure and stirring rate 1500 rpm. An oil product was extracted with dichloromethane in a Soxhlet apparatus. Screening tests show that Fe_2O_3 has a negligible effect on oil yield and zeolitic support is unsuitable for hydrogenation of lignin because its channel system makes lignin

difficult to access the active site. Pd/C exhibits highest activity among Ni/Mo/Al₂O₃/SiO₂ > Raney Ni, = NiO/MoO₃/Al₂O₃/SiO₂ in terms of oil yield. The composition of oil depends on the catalyst. Mo catalyst mainly gives monophenols while Pd/C produced ethylcyclohexanones and catechols, and so on. Ni/Mo was found possessing a high demethoxylating power, and Pd or noncatalyst tests showed demethylation to catechols. It was also noted that sulfided NiMo was more active than the oxidized form when much higher oil yield was observed with kraft lignin than that from organocell lignin. The acetyl group in acetosolv lignin lowered the oil yield due to blockage phenomena. Surprisingly, oil composition from different types of lignin shows no difference.

Iron compounds (FeS or FeSO₄) used in coal liquefaction were also examined for HTL of biomass to oil. Xu and Etcheverry (2008) treated 1 g of Jack pine wood with particle size smaller than 20 mesh (~0.8 mm) in a 14-mL reactor with 5 wt % catalyst and 13 mL ethanol in the presence of 2.0–10.0 MPa hydrogen. Generally, oil yield (15%–35%) increases with reaction time of 15–60 minutes, temperature of 200–260°C, and initial H₂ pressure of 2.0–10.0 MPa without a catalyst. At the supercritical condition of ethanol, 45% oil yield can be obtained at 350°C, but more char formed at a temperature more than 300°C. In the presence of 5% FeS or FeSO₄, the oil yield shows no significant improvement. It was noted that ethanol consumption and contribution to oil were considered.

An organic solvent layer may extract oil molecules from the aqueous phase during HTL in a two-layer reaction system. Miller and Fellows (1981) reported that wood or cellulose can be almost totally converted to liquids or gases at 350°C in pressurized phenol and water with catalyst. In a typical reaction, 2 g biomass mixed with 2 g phenol, 2.5 g water and 0.5 g catalyst was heated to 350°C for a few minutes to several hours in a pressurized glass vessel. The phenol in a two-layer reaction system, which can be produced from lignin and recycled, intends to provide a solvent to slow down the higher order solid state condensation reaction. The recovered yield of neutral product was 0.55–0.76 g per 2.5 g dry aspen. About 3% aromatic hydrocarbons, including toluene, ethyl benzene, and xylene were found in nonphenolic products when zinc chloride and nickel were used with hydrogen. In addition, the observed recoveries showed that a net phenol could be produced.

Except for phenol, other organic solvents such as acetone, methyl ethyl ketone, 1-, and 2-propanol, and 1-butanol were used for biofuel production with marked effect on the direct formation of a fluidized product. Ogi et al. (1990) examined the role of butanol solvent in direct liquefaction of wood. The wood chips in 80 mesh were heated with water, sodium carbonate, and organic solvent in an autoclave under nitrogen pressure. They observed that there were three phases, that is, butanol layer, water layer, and tar-like product on the reactor wall when butanol was used as a solvent. (It is interesting to know that t-butanol is different from 1-, 2-, and i-butanol, which mixes with water freely.) Blank tests indicated that butanol, especially t-butanol, degraded under experimental conditions of 270°C, 90 atm, and 60 minutes retention time. They found that isomers of butanol have no effect on oil yield as high as 45%–55% assuming that no isomer was converted to oil. The function of sodium carbonate was thought to be an agent inhibiting hydrolysis of biomass. The use of hydrogen donors is believed to be one of the most efficient ways to reduce these undesirable reactions. They clarified that butanol did not function as a hydrogen donor solvent, but only acted as an extraction solvent/stabilizer, in which undesirable reactions such as repolymerization were retarded.

Instead of direct TCC of biomass to oil in one reactor, a two-stage HTL was employed to produce the alternative oil product from lignocellulosic biomass. Román-Leshkov et al. (2007) developed a process to produce dimethylfuran for liquid fuels from biomass-derived

carbohydrates. The sugar solution was converted to HMF in high yield by the acid-catalyzed dehydration of fructose in a biphasic reactor using a low boiling point solvent (e.g., butanol) that continuously extracts the HMF product with NaCl presented in the aqueous phase. The extracting solvent containing HMF was then purified in an evaporator at low temperature (e.g., 89.9°C). Next, HMF is converted to dimethylformamide (DMF) over a copper-based catalyst such as CuCrO_4 or CuRu/C by hydrogenolysis. Finally, DMF was separated from the solvent and intermediates via a distillation process. Sugar dehydrates at 180°C for 3 minutes with ~75% conversion and 5 wt % HMF can be converted to DMF with 61%–71% yield over CuRu/C catalyst in a flow reactor at 220°C, 6.8 bar hydrogen, and feed rate of 0.2 cm³/min.

Minowa et al. (1998) applied the HTL process to 18 kinds of agricultural and forest residues in Indonesia. Tests were run in a 300 mL stainless steel autoclave with a magnetic mixing, at 300°C, and 30 minutes retention time. N_2 was used as the initial gas and added to 3 MPa at the beginning. Five wt % Na_2CO_3 was used as the catalyst and acetone was used to extract the oil product. Oil yields were in the range of 21%–36%, depending on the species and parts of feedstock. All oils had almost the same elemental properties, C ~70%, H ~7%, N <1%, O ~20%. Heating values were around 30 kJ/g and viscosity was greater than 105 mPa.s. Gas yields were around 20%, consisting mainly of CO_2 with a range of 78–86 mol %. Other gases included CO (11–19 mol %) and H_2 (~2 mol %). More than 35% of the energy in raw materials was recovered in the form of oil, and for some materials, this number was as high or more than 50%. Residue also had energy content, indicating it is possible to use residue as a process energy source, especially for coconut husk and oil-palm shell.

A high correlation between lignin and residue was observed (Minowa et al. 1998). The phenoxy radicals could be formed from the lignin under high temperatures, and a higher lignin content resulted in a higher residue yield by condensation and repolymerization.

The correlation between lignin and residue was also studied by Demirbas (2000) and a similar conclusion was drawn. In his study, nine species of biomass with different lignin contents were thermohydrolyzed in an autoclave with and without a KOH catalyst (20 wt %). All tests were performed in a 250 mL cylindrical autoclave. Tests were run at 575 K over 30 minutes, using N_2 as the initial gas. There was a strong correlation between lignin content and oil yield. With increasing lignin content, the oil yield decreased and the char yield increased.

Nelson et al. (1984) studied the mechanisms of direct liquefaction of cellulose. At 250–400°C, a pressure up to 20.7 MPa, and with the presence of Na_2CO_3 , pure cellulose was converted in a 300-mL autoclave, to a mixture of phenols, cyclopentanones, and hydroquinones as well as other components. At 300°C for 1 hour, most of the oil components are present in amounts of 0.1 wt % or less in the oil, which makes it very complicated to analyze the oil product. Phenolic products have been observed as products of the alkali treatment of saccharides. Biacetyl and acetoin are precursors for aromatic components during cellulose liquefaction. A scheme of the formation of biacetyl and acetoin from cellulose was discussed. Faster heating rates would be useful to reduce the inevitable degradation and recombination of the initial products. The use of alkaline catalysts at 300°C was shown to shift the mechanism from one involving aqueous pyrolysis (predominant furan formation) to one incorporating aldol and related condensations.

Russell et al. (1983) used selected aldehydes and ketones which might have formed from cellulose degradation as model compounds to study the formation of aromatic compounds during cellulose liquefaction, under the same conditions as those of cellulose liquefaction. Many of the same aromatic compounds were formed from these reactions as were found in

cellulose derived oils. The condensation and cyclisation of aldehydes and ketones is apparently involved in the formation of aromatic compounds in cellulose liquefaction oils. Eight aromatics were identified in both cellulose oils and model compound products. Mechanisms were proposed for five of the eight aromatics.

Maldas and Shiraishi (1997) studied the liquefaction of biomass in the presence of phenol and water, using alkalis and salts as catalysts. A closed pressure-proof tube was used as a reactor at 150–250°C, 0–90 minutes reaction time. A higher temperature was required to reduce residue. A reaction time of 45 minutes was sufficient to maintain low residue and to keep constant other parameters, such as nonreacted phenol, combined phenol, and pH of liquefied mixtures. Aqueous alkali was more effective than water for the liquefaction of biomass in phenol. The ultimate pH of liquefaction was always acidic whether the starting pH was alkaline or acidic. Dissolution of wood varied significantly with the variation of reaction conditions, for example, pH, temperature, and nature of metal ions.

Inoue et al. (1999) used ammonia and cellulose as feedstock to study the effect of nitrogen/carbon ratio in the feedstock on the oil production. Ammonia water of 25 wt % and microcrystalline cellulose were charged into 500 mL autoclave, and liquefied at 300°C, 2 MPa N₂ initial pressure, and 1 hour retention time. Alkali acted as a catalyst for hydrolysis of cellulose into small fragments and for prevention of undesirable reactions, such as polymerization. Ammonia acted both as reactant and a basic catalyst. The liquefaction process resulted in the creation of C-N bonds. Yield and nitrogen content of the oil increased with increasing N/C ratio. Excess ammonia did not react with cellulose under high N/C ratio. Productive mechanism of nitrogen-containing oil from protein-containing feedstock might be the decomposition of proteins to water-soluble materials, which are then combined and converted into oil. Not only amine and amide were contained in the oil, but also heterocyclic compounds. Oil derived from this liquefaction consists of aldehydes, ketones, and aromatic compounds, containing many unidentified compounds.

Conversion of Algae to Biofuel

If biomass were grown for energy to an amount equal to that consumed during their any given production period, there would be no net buildup of CO₂ in the atmosphere (Gao and Mickinley 1994). Microalgae are particularly promising biomass species because of the high growth rate and high CO₂ fixation ability compared to plants (Tsukahara and Sawayama 2005).

The earliest idea focused on producing methane gas from microalgae. The concept of producing fuel by using microalgae as a source was reported by Meier (1955). Golueke and Oswald (1959) presented the concept of using microalgae as a substrate for anaerobic digestion, and the reuse of the digester effluent as a source of nutrients. They realized these concepts by using a large pond (40 pa) to grow microalgae, then the microalgae was digested to methane gas for producing electricity. The gas production by the digester averaged about 10 ft³ per lb of volatile matter introduced. The methane content of the gas varied from 68% to 74%. The maximal efficiency attained by the algal culture was 3%, whereas the maximal overall efficiency of the entire conversion unit was approximately 2% (Golueke and Oswald 1959). From the 1970s, the National Science Foundation-Research Applied to National Needs Program (NSF-RANN) started to support laboratory studies of microalgae fermentations to methane gas (Uziel et al. 1975). Six microalgae species were studied, approximately 60% of microalgae biomass energy could be converted to methane gas. It was found that the rate of biogenic methane gas production by the marine strain methanogenic bacteria at 50% wet

algal thalli amendment was greater by 33.4% in comparison with results of the freshwater cattle manure strain methanogenic bacteria under similar experimental conditions. The proportion of methane gas content in this biofuel gas was 58%, while the remaining gases are CO₂ (major portion), H₂S, NH₃, N₂, and O₂ (Silvalingam 1982).

Many microalgae, in particular species classified as “green algae,” produce hydrogen after a period of anaerobic conditions in the dark, during which the hydrogenase enzyme was activated and synthesized, and small amounts of hydrogen production were observed (Das and Veziro lu 2001). Green algae are probably better for hydrogen production than cyanobacteria (blue-green algae) whereas the latter uses more energy-intensive enzymes, ATP-requiring nitrogenase for the production of H₂ (Lee and Greenbaum 1997).

Methane production from microalgae became the basis and motivation of the U.S. DOE's program to develop renewable transportation fuels from microalgae, which started in 1978 and ended in 1998. This program mainly focused on the production of biodiesel from high lipid-content microalgae grown in ponds, utilizing waste CO₂ from coal fired power plants. Biodiesel is an alternative fuel produced from triglycerides and fatty acids present in naturally occurring fats and oils. Traditional oil crops such as corn, soybeans, canola, coconut, and oil palm cannot adequately contribute to replacing petroleum derived from liquid fuels in the foreseeable future due to their relatively low oil yield per hectare compared with microalgae. For example, 30% oil (by wt) of microalgae has an oil yield of 58,700 l/ha and 70% oil (by wt) of microalgae has an oil yield of 136,900 l/ha. By comparison, corn and soybeans have only an oil yield of 172 l/ha and 446 l/ha, respectively (Chisti 2007). Hu et al. (2008) reported that based upon the photosynthetic efficiency and growth potential of microalgae, theoretical calculations indicated that annual oil production of larger than 30,000 L or about 200 barrels of algal oil per hectare of land may be achievable in mass culture of oleaginous algae (algal species have been found to grow rapidly and produce substantial amounts of triacylglycerols or oil). This value was 100-fold greater than that of soybeans, a major feedstock currently being used for biodiesel in the United States.

Another unique benefit of using microalgae to produce biodiesel is that it will not compromise production of food, fiber, and other products derived from crops. Xu et al. (2006) used n-hexane to extract large amounts of microalgal oil from *Chlorella*, which the crude lipid content is about 55.2%. Then the microalgal oil was transformed into biodiesel by acidic transesterification. The biodiesel was characterized by a high heating value of 41 MJ/kg, a density of 0.864 kg/L, and a viscosity of 5.2×10^{-4} Pa·s at 40°C (Xu et al. 2006).

Pyrolysis with different solvents and re-agents were conducted on algae-protein (Goldman et al. 1980). The reactions yielded rather low conversions in the presence of water in spite of the existence of carbonates and catalysts, for example, nickel sulfate. The presence of benzene improves the yield and the presence of a mixture of K-Mg-Mn salts was beneficial for such a reaction. The nitrogen content of liquid oil decreased in the presence of carbonates and other catalysts. The maximum amount of protein converted into liquid oil was 27% by weight for algae-proteins containing 5.7 wt % nitrogen.

Lipid content in microalgae was considered as the most important component for yielding biofuel, such as biodiesel and other forms of oil. Peng et al. (2001a) studied the pyrolytic characteristics of *Chlorella protothecoides*. *Chlorella protothecoides* were pyrolyzed at the heating rates of 15, 40, 60, and 80°C/min up to 800°C. The pyrolysis reactions mainly took place between 160–520°C with a volatile yield of about 80%. The devolatilization stage consisted of two main temperature zones (I and II) with a transition at 300–320°C. The researcher found that crude lipid in cells decomposed at Zone II while other main components at Zone I, which might indicate that more energy input for lipid pyrolysis seems needed in

comparison with other main components (Peng et al. 2001b). In another study, two kinds of high protein and lipid contents microalgae, *Cyanobacterium Spirulina platensis* (SP) and green alga *C. protothecoides* (CP) were pyrolyzed at the heating rates of 15, 40, 60, and 80°C/min up to 800°C in the thermogravimetric analyzer to investigate their pyrolytic characteristics. The results showed the value of activation energy for CP pyrolysis was lower than that of SP, and the char in final residue of CP was 2%–3% less than that of SP, which indicated CP was preferable for pyrolysis over SP (Peng et al. 2001).

Fast pyrolysis (a sweep gas [N₂] flow rate of 0.4 m³/h, and a vapor residence time of 2–3 s) was used to treat *C. protothecoides* to produce bio-oil. The highest yield of bio-oil is 57.9%, at an operating temperature of 450°C. This yield is 3.4 times higher than that from autotrophic cells also treated by fast pyrolysis. After reaction, the total liquid products were composed of an aqueous and an oil phase. The oil was fractionated using column liquid chromatography, and separated into n-hexane soluble and n-hexane insoluble compounds. The bio-oil was characterized by a much lower oxygen content, with a higher heating value (41 MJ/kg), a lower density (0.92 kg/L), and lower viscosity (0.02 Pa·s) compared to those of bio-oil from autotrophic cells and wood. These properties are comparable to fossil oil (Miao and Wu 2004).

Algal lipid or even the whole algae could be pyrolyzed to a similar, high-octane, aromatic gasoline product slate when passed over HZSM-5, a medium-pore, shaper-selective, acid catalyst. The first of these results was reported by Milne and Evans (1987). The same authors carried out exploratory studies of the pyrolysis and zeolite conversion of whole algae and their major components. Four species of microalgae were pyrolyzed: *Chaetoceros muelleri* var. subsalsum, *Monoraphidium minutum*, *Naviculus Saprophilla*, and *Nannocloropsis* sp. However, the results of whole algae were ambiguous due to very high mineral matter content (10%–50%) and unknown proportions of water, lipids, and other organic components of the exact sample used (Milne et al. 1990).

One of the shortcomings of using pyrolysis to convert microalgae is due to the high moisture content of microalgae. The large amount of energy consumed to vaporize the water during the pyrolysis process was considered as a negative effect of this method (Minowa et al. 1995a). Dote et al. (1994) performed a liquefaction of *Botryococcus braunii*, with and without sodium carbonate as a catalyst. High-quality oil was obtained, which was more than the content of hydrocarbons in *B. braunii* (50 wt % db), in a yield of 57–64 wt % at 300°C. The oil was equivalent in quality to petroleum oil (Dote et al., 1994). The properties of the oil obtained from *B. braunii* were clarified by the same research group (Inoue et al. 1994). The oil was fractionated into three fractions by silica gel column chromatography and analyzed to determine its composition. The yields of the three fractions based on organics were 5% of lower molecular weight hydrocarbons (MW = 197–281), 27.2% of botryococcenes (MW = 438–572), and 22.2% of polar substances (MW = 867–2209). The maximum recovery (78%) of botryococcenes in the liquefied oil was achieved at 200°C with the use of a catalyst.

Minowa et al. (1995b) used HTL to convert *Dunaliella tertiolecta* (78.4% moisture content) into oil at around 300°C and 10 MPa. The *D. tertiolecta* was cultured batchwise in an open tank of 10-L capacity at continuous light of 20,000 lux, temperature of 27°C, and bubbling air with 3% CO₂. Then the algal cells grown for HTL were harvested by a centrifugal separator. Some Na₂CO₃ (0–5 wt % of the dry solid in the algal cells) was used as additive. However, the results showed it had no catalytic effect on either the oil yield or its properties. Nitrogen was introduced to purge the residual air in the autoclave. To prevent water from vaporizing, additional nitrogen was added to 3 MPa. Several reaction temperatures were tried: 250, 300, and 340°C. The retention time (holding time) was from 5 minutes to 60 minutes.

The gas phase was primarily CO_2 . Besides the gas phase, the reaction mixture consisted of a tar-like material and a water phase. The tar-like material floated on the surface of the water phase in all experiments and was easily separated. The oil was extracted from the reaction mixture by dichloromethane. Then the dichloromethane was evaporated from the extract at 35°C under reduced pressure, yielding a dark-brown viscous material, which was referred to as the oil. The oil was obtained in the range of 31%–44% (average 37%) on an organic basis. The oil yield exceeded the algal cells crude fat content (20.5%). The reaction parameters, including reaction temperature, holding time, and sodium carbonate addition, had no significant effect on the oil yield. However, the author thought the properties of oil strongly depended on the reaction temperature. The viscosity decreased and the heating value increased slightly with a rise in temperature. The carbon and hydrogen content tended to increase with temperature increases. The oil obtained at a reaction temperature of 340°C and holding time of 60 minutes had a viscosity of 150–330 mPa·s and a calorific value of 36 MJ/kg. These values were comparable to those of No. 3 fuel oil in JIS (50–1000 mPa·s, about 40 MJ/kg). The results of the energy consumption ratio (ECR) indicated the liquefaction was a net energy producer (Minowa et al. 1995b).

Metal catalysts had been used in microalgae liquefaction. Matsui et al. (1997) investigated the liquefaction of *Spirulina*, a high-protein microalgae in various organic solvents or water under hydrogen, nitrogen, or carbon monoxide in the temperature range 300 – 425°C , using $\text{Fe}(\text{CO})_5$ -S catalyst. Among the solvents of tetralin, 1-methylnaphthalene, toluene, and water, it seemed more favorable for liquefaction of *Spirulina* to take place in water. After reaction, tetrahydrofuran (THF) was used to extract the production. Then the THF-soluble fraction was further separated into hexane-insoluble and hexane-soluble fractions by precipitation into hexane. The hexane-soluble fraction was denoted as oil in this research. Liquefaction of *Spirulina* at 300 – 425°C under hydrogen gave more than 90 wt % conversion and 60 wt % oil yield. Addition of the $\text{Fe}(\text{CO})_5$ -S catalyst increased the oil yield from 52.3 wt % to 66.9 wt % at 350°C for 60 minutes in tetralin. Liquefaction in water gave an oil yield as high as 78.3 wt % at 350°C even under nitrogen without a catalyst. Liquefaction in toluene gave oil fractions having a heating value of 32–33 MJ/kg, but products obtained in water, containing large amounts of oxygen, were estimated to have a lower heating value of 26 MJ/kg (Matsui et al. 1997).

The microalgae and coal were co-liquefied with the presence of coal liquefaction catalysts (Ikenaga et al. 2001). Dried samples of *Chlorella*, *Spirulina*, and *Littorale* were used as microalgae and Australian Yallourn brown coal and Illinois No. 6 bituminous coal were employed. Commercial iron pentacarbonyl ($\text{Fe}(\text{CO})_5$), trisruthenium dodecacarbonyl ($\text{Ru}_3[\text{CO}]_{12}$), and molybdenum hexacarbonyl ($\text{Mo}(\text{CO})_6$) were employed as catalysts. The co-liquefaction was carried out under pressurized H_2 in 1-methylnaphthalene at 350 – 400°C for 60 minutes. Co-liquefaction of *Chlorella* with Yallourn coal was successfully achieved with excess sulfur to iron ($\text{S}/\text{Fe} = 4$). The conversion and the yield of the hexane-soluble fraction were close to the values calculated from the additivity of the product yields of the respective homo reactions. All three catalysts were effective for the co-liquefaction of microalgae with coal. Some 99.8% of conversion and 65.5% of hexane soluble fraction were obtained at 400°C with $\text{Fe}(\text{CO})_5$ at $\text{S}/\text{Fe} = 4$, when the 1:1 *Chlorella* and Yallourn coal were co-liquefied. In the co-liquefaction of *Chlorella* with Illinois No. 6 coal, the oil yield was close to the additivity of the respective reaction with $\text{Fe}(\text{CO})_5$ -S, even at $\text{S}/\text{Fe} = 2$. $\text{Mo}(\text{CO})_6$ catalyst ($\text{S}/\text{Mo} = 4$) was the most effective for the respective homo-liquefactions of *Chlorella* and Yallourn coal. When *Littorale* and *Spirulina* were used as microalgae, a similar tendency was observed with the iron catalyst.

Microalgae culturing conditions and the fixation of CO₂ during the microalgae growth were studied in order to find the optimal condition to harvest large amounts of algae. It was also useful to investigate the energy balance and CO₂ mitigating effect of a liquid fuel production process from microalgae using HTL. Kishimoto et al. (1994) studied the CO₂ fixation during the microalgae growth and oil production of microalgae by using thermochemical liquefaction. The green microalga *D. tertiolecta* AHTP 30929, which contained 10% glycerol, was used throughout the study. The effects of saline, light intensity, and CO₂ concentration on microalgae growth were investigated. The details of the liquefaction were not mentioned in this paper, but the authors concluded that the heavy oil yield from the liquefaction was 35.6%. The contents of the chemical elements were as follows: carbon 73%, hydrogen 9%, nitrogen 5%, and oxygen 13%. The heating value of the heavy oil was 34.7 MJ/kg, which was almost the same as C heavy oil. The viscosity of the oil was 860 mPa·s, the same as that of castor oil. The nitrogen content was higher than that of ordinary petroleum.

Continuous culturing of the *B. braunii* Berkeley strain in secondarily treated sewage (STS) was conducted and then liquefied by Sawayama et al. (1995). *B. braunii* grew continuously for a period of over 1 month at a growth rate of 200 mg dry weight per liter water per week and the algal cells fed on STS containing 49% oil as the hexane soluble fraction. Liquefaction of microalgae cells was conducted in a 300 mL autoclave. Wet cells (30 g wet weight, 92% moisture content) were charged to the autoclave with 5 wt % or without sodium carbonate. N₂ was used as the initial gas for preventing water from vaporizing. The autoclave was heated to 200, 300, and 340°C, with a retention time of 1 hour. Oil was extracted with dichloromethane from the reaction mixture. The maximum yield of oil obtained by liquefaction was 64 wt % on a dry basis at 300°C with a sodium carbonate catalyst. The yield of the hexane soluble fraction was 97 wt % compared with that in the feedstock algal cells. The heating value of the liquefied oil obtained from this reaction was 49 MJ/kg and the viscosity was 64 mPa·s at 50°C.

Different microalgae are not the same in producing oil through liquefaction. The data of liquefaction of microalgae *B. braunii* and *D. tertiolecta* were collected and compared, and then the energy balance of the reactions was calculated by Sawayama et al. (1995). Liquefaction was performed using a stainless steel autoclave with 100 or 300 mL capacity using 0–5 wt % and Na₂CO₃ as a catalyst. After purging with nitrogen, the autoclave was charged with nitrogen at 2–3 MPa. The reaction temperature was 300°C. The reaction mixture included a gas mainly composed of CO₂, a tar-like material that sank to the bottom, a water phase, and an oil-like material that floated on the surface of the water phase. Solvent extraction with CH₂Cl₂ or acetone was performed to separate the oil from the reaction mixture. The yield of oil was determined as a percentage by weight of the organics in the original material. Based on the energy calculation and comparison, the yield of liquid fuel produced from *B. braunii* and its lower heating value were high compared with those of *D. tertiolecta*; therefore, the energy inputs for cultivation and separation of *B. braunii* were calculated to be smaller than those of *D. tertiolecta*. The energy input for fertilizers of *B. braunii* was also smaller than that of *D. tertiolecta*. Therefore, the hydrocarbon-rich microalga, *B. braunii*, could be more suitable for liquid fuel production using thermochemical liquefaction compared with *D. tertiolecta*. The authors also concluded: if a 100-MW thermal plant using coal would be replaced by liquid fuel produced from *B. braunii*, the quantity of CO₂ mitigation could be 1.5×10^5 t/yr and 8.4×10^3 ha of microalgal cultivation area could be necessary (Sawayama et al. 1999).

To find the most suitable operational conditions of HTL of microalgae, Yang et al. (2004) used the *Microcystis viridis* strain as feedstock. The series of experiments were conducted

under the following various conditions: catalyst (Na_2CO_3) loading rate of 0 and 5 wt %; reaction temperature of 300 and 340°C; and holding times of 30 and 60 minutes. The initial operational pressure was designed at 3 MPa of nitrogen, and the maximum pressure of the autoclave was 10–20 MPa in order to decrease water evaporation. The reaction mixture was extracted with chloroform to recover the oil by evaporating chloroform at 40°C. The aqueous phase and insoluble mixture were separated by filtration. The chloroform insoluble fraction remaining on the filter paper was dried at 105°C for one day to obtain a solid residue. The evolved gas was transferred to a sampling bag and composition was determined by gas chromatography. The oil yield was defined as the ratio of the weight of oil products after liquefaction to the weight of organic matter in feedstock. The energy yield was defined as the ratio of the weight of C and H in oil products after liquefaction to the weight of C and H in feedstock. After investigating the effects of the reaction parameters, such as retention time, reaction temperature, and load of the catalyst, the maximum 33% oil yield (energy yield of around 40%) was obtained with the 30-minute holding time, reaction temperature of 340°C, and alkali catalyst dosage of 5 wt %. The heating value of the obtained oil was 31 MJ/kg, less than that of heavy oil (40 MJ/kg). The elemental composition of liquefied oil was composed of 62% carbon, 8% hydrogen, 8% nitrogen, and 2% sulfur. The liquefied oil contained n-alkane of C_{17} – C_{18} hydrocarbon as a main component of the saturated compounds, so typical aromatic compounds of heavy oil, such as n-naphthalene and n-dibenzothiophene were found in liquefied oil, and it was considered that the liquefied oil should be classified as heavy oil. The gas consisted primarily of CO_2 and methane. The total nitrogen in the aqueous phase ranged from 998 to 1157 mg/L, and half of the total nitrogen was detected as ammonia nitrogen. The total phosphate in the aqueous phase ranged from 2.47 to 5.38 mg/l.

After microalgae liquefaction, other forms of biofuel—such as biodiesel—could be obtained by further extraction. Based on the efficiency of biodiesel extraction, Aresta et al. (2005) compared HTL of algae with the extraction using supercritical carbon dioxide (sc- CO_2). Green alga *Chaetomorpha linum* was used as the feedstock. Almost 20 g of fresh, washed thalli of *C. linum* were placed into the glass reactor which was then put into the autoclave. The latter was closed under N_2 atmosphere to purge the residual air and 3.0 MPa of N_2 . The autoclave was then heated to the desired temperature (250, 300, 350, and 395°C) for 1 hour. The reaction mixture was recovered and treated with CH_2Cl_2 . Then an organic and an aqueous/solid suspension were separated. From the organic solution, after evaporation of the solvent under controlled conditions, an amber-yellow oily liquid was obtained. The aqueous solution was separated and a solid was recovered by centrifugation. For the sc- CO_2 extraction, the thalli of alga were dried for 5–8 days at room temperature, 3–5 g of dried algae were milled in liquid nitrogen (5 mL) to break the cellular wall in order to increase the extraction yield. A 0.5–1 mL of methanol was used as a co-solvent to improve the efficiency of the extraction. In both liquefaction and sc- CO_2 extraction, the oils obtained were treated to convert all fatty acid (FA) components into the mono-methyl-esters (Biodiesel). Then the extracted material was analyzed quantitatively by GC and qualitatively by GC-MS. For liquefaction, increasing the temperature for the total amount of oil extracted from the algae increased reaching a plateau between 350–395°C (about 80 mg/g dry wt). Increasing the temperature affected the amount of fatty acid extracted. However, at higher temperatures, some oil decomposed, resulting in a decrease of the recovered amount of extracted fatty acid. After comparing the results from the sc- CO_2 extraction, the authors implied that HTL seemed to be more efficient from the quantitative point of view.

Summary

HTL mimics the natural process of fossil fuel formation from biomass feedstocks. In that sense, it holds great promise as a renewable energy source, especially for liquid fuel. Although great effort has been made since the 1970s, the results have been intermittent due to the low energy price since the 1980s. It is time once again that we need to revisit the science and technology of HTL, together with other renewable technologies.

HTL of biowaste materials into liquid fuel holds several unique advantages. First, it safeguards national security—by using biowaste and producing algae within dispersed local communities, the geopolitical threat from the required importation of petroleum can be neutralized. Second, it has a net-zero carbon emission—bio-waste materials are renewable, typically a product or byproduct of photosynthesis. Third, it does not compete with the food supply—bio-waste is often a negative-cost feedstock. In the event of algae growth within or at nearby waste treatment facilities and animal farms, HTL will not compete with the supply of farmland or food.

Acknowledgment

The authors wish to acknowledge several graduate students, Rong Dong, Zhichao Wang, and Guo Yu, for help on the literature search on the topics of HTL of different feedstocks.

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Chapter 11

Detoxification of Lignocellulosic Hydrolysates

Bin Wang and Hao Feng

Abstract

Typically, lignocellulosic biomass must be deconstructed into monosaccharides for efficient conversion into biofuel by fermenting microorganisms. Natural protection mechanisms of plants against foreign intrusions such as disease-causing bacteria and viruses create obstacles to biomass deconstruction and economical production of biofuel from biomass. Large-scale production of biofuel from lignocellulosic biomass, therefore, is still a challenge to both microbiologists and engineers. A principal problem associated with deconstruction of biomass by chemical, enzymatic, or biological process is the generation of microbial inhibitory chemicals. Presence of these inhibitory compounds in the lignocellulosic hydrolysates inhibits growth and biofuel production by fermenting microorganisms. An additional processing step called detoxification, therefore, has often been introduced to remove these microbial inhibitors from hydrolysates as a way of mitigating their negative effects on the fermenting microorganisms. Efforts have been made over the years to develop effective detoxification processes with chemical, physical, or biological methods. This chapter, detoxification of lignocellulosic hydrolysates, will focus on the technical aspects of lignocellulosic hydrolysates detoxification approaches and their potential applications in biomass-to-fuel production.

Toxic Compounds in Lignocellulosic Hydrolysates

Toxic compounds in lignocellulosic hydrolysates can be divided into four major groups: sugar degradation products, lignin degradation products, compounds derived from lignocellulose structure, and heavy metallic ions (Mussatto and Robert 2004). Typical inhibitory compounds include carboxylic acids, aldehydes, furans, and phenolics (Zaldivar and Ingram 1999; Palmqvist, and Hahn-Hägerdal 2000b; Oliva et al. 2003; Varga et al. 2004). The first step in the conversion of biomass to fermentable sugars is pretreatment. A pretreatment process uses mainly physical and chemical methods to increase surface area; decrystallize cellulose;

remove sheathing on cellulose by hemicellulose and lignin; alter lignin structures; and sometimes partially remove lignin to improve both the rate of enzymatic hydrolysis and the yield of monosaccharides (Mosier et al. 2005a). Different pretreatment methods, including steam explosion (Öhgren et al. 2005), dilute sulfuric acid (Lloyd and Wyman 2005), hot water (controlled pH) (Mosier et al. 2005b), ammonia fiber/freeze explosion (AFEX; Teymouri et al. 2005), ammonia recycle percolation (ARP; Kim and Lee 2005), lime (Kim and Holtzapple 2005), ionic liquid (Dadi et al. 2006, 2007), and peroxide (Saha and Cotta 2006), among others, have been proposed and tested for deconstruction of a variety of lignocellulosic biomass. The pretreatment of lignocellulosic biomass by acids or water often results in the degradation of cellulose, hemicellulose, and lignin (Figures 11.1–11.3). These degradation compounds exert inhibitory effects on fermentation microorganisms.

Enzymatic hydrolysis of lignocellulosic biomass may also release inhibitors from biomass (Figure 11.4). The arabinoxylan from the primary cell walls of gramineous monocots, for example, is esterified with ferulic and *p*-coumaric acids. Varga et al. (2004) found that

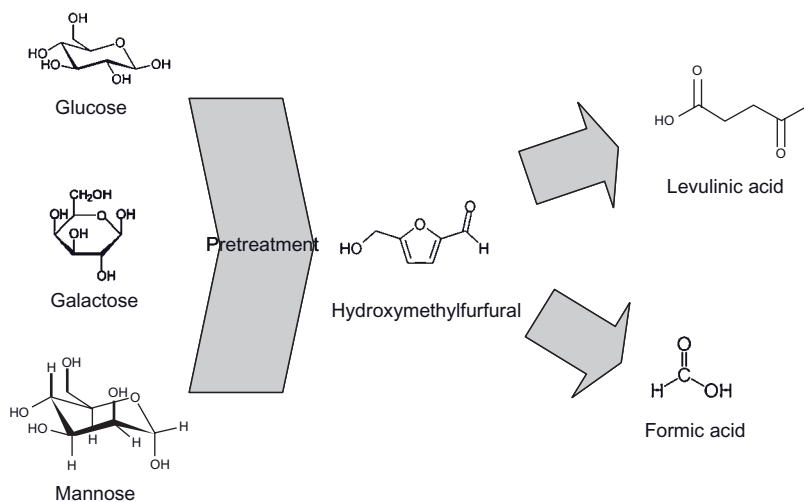


Figure 11.1. Inhibitors from hexose degradation (Palmqvist et al. 2000a).

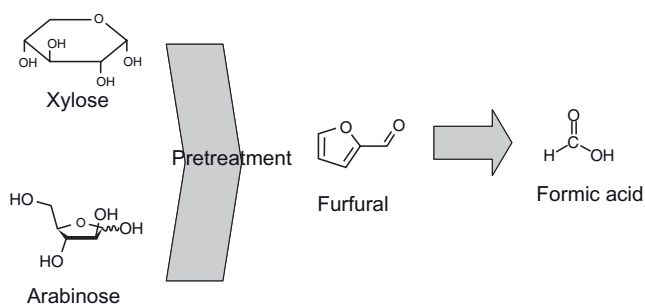


Figure 11.2. Inhibitors from pentose degradation (Zaldivar et al. 2001; Usuki et al. 2008).

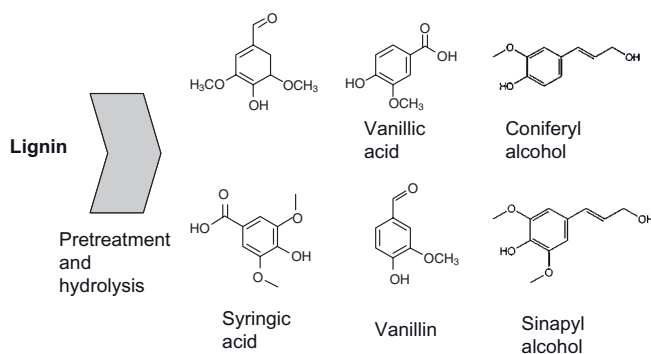


Figure 11.3. Inhibitors from lignin degradation (Klinke et al. 2004; Chang 2007).

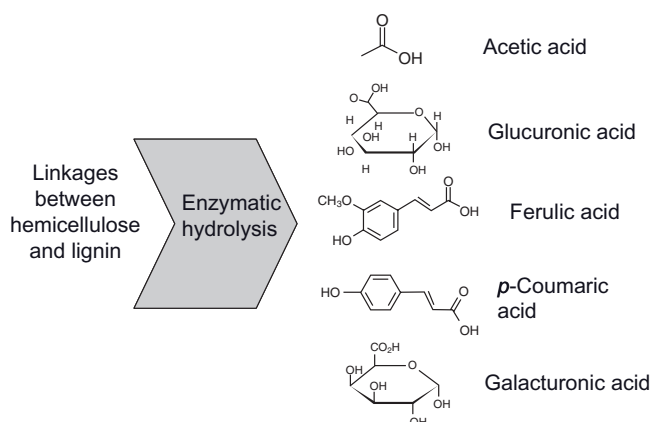


Figure 11.4. Inhibitors from linkages between hemicellulose and lignin (Klinke et al. 2004).

arabinoxylan, coumaric acid, and ferulic acid are significant components of solubilized corn stover hemicellulose produced during simultaneous saccharification and fermentation (SSF). These acids from biomass structure are toxic to fermentation microorganisms. When 0.3 g/L of *p*-coumaric and ferulic acids were incorporated into a fermentation medium, the acetone–butanol–ethanol (ABE) production by *Clostridium beijerinckii* BA101 decreased significantly (Ezeji et al. 2007).

Heavy metal ions (iron, chromium, nickel, and copper) can originate from corrosion of hydrolysis equipment (Figure 11.5). Although they are not always produced in large quantities, they can have some toxic effect on the alcoholic fermentation microorganisms (Mussatto and Robert 2004).

The toxic effects of various inhibitory compounds on the growth response of ethanologenic *Escherichia coli* were found to be in the following order: aldehydes > organic acids > alcohols (Zaldivar and Ingram, 1999; Zaldivar et al. 1999, 2000). When plotting series of separate functional groups of phenol, aldehydes, ketones, and acids, a correlation between the hydrophobicity and inhibition of volumetric ethanol productivity was reported.

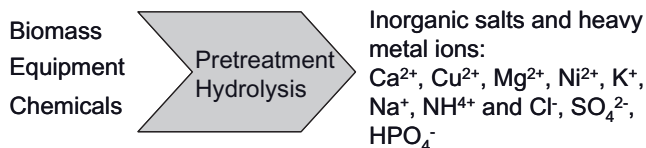


Figure 11.5. Inorganic salts and heavy metal ions (Klinke et al. 2004; Mussatto and Roberto 2004).

The more hydrophobic the compound was, the more the inhibition was evident. The phenolics were reported to be among the most toxic compounds to fermentation microorganisms (Larsson et al. 2000; Klinke et al. 2004).

Detoxification Methods

To overcome the toxic effects of inhibitory degradation products of lignocellulosic biomass, many detoxification methods have been investigated. The focus has been placed on removing the inhibitory compounds from the hydrolysates, modifying the inhibitory compounds, or improving the resistance of the fermenting microorganisms to the toxic effects of the inhibitory compounds (Palmqvist and Hahn-Hägerdal 2000a; Pienkos and Zhang 2009). The detoxification methods can thus be generally divided into three categories: chemical, physical, and biological methods (Table 11.1).

Physical Detoxification Methods

Steam Stripping

Steam stripping, also known as steam distillation, is a process of removing temperature-sensitive compounds that cannot be separated by normal distillation due to decomposition at high sustained temperatures. It has been used to remove various organic contaminants from process plant waste water streams. Steam stripping can also be used to detoxify lignocellulosic hydrolysates (Yu et al. 1987). Leonard and Peterson (1947) used steam stripping to remove inhibitory volatiles, such as furfural and acetic acids, from hydrolysates of maple and spruce. Maddox and Murray (1983) passed steam through hydrolysates of *Pinus radiata* to achieve a liquor temperature of 90°C for 15 minutes. Treatment of the hydrolysates by steam stripping followed by passage of the hydrolysates through activated carbon led to successful fermentation, but the treatment procedure caused about 30% sugar losses. The high stripping temperatures allow the removal of heavy and soluble organic compounds. The only waste generated in steam stripping is a small amount of concentrated organics. These organic wastes are easily processed by incineration, biological treatment, or recycling. However, steam stripping is not a good solution for hydrolysates that contain nonvolatile phenolics with high boiling points.

Evaporation

Evaporation when used as a detoxification method removes only volatile inhibitors. However, in previous studies, the volatile compounds did not affect either the enzymatic hydrolysis or

Table 11.1. Summary of detoxification methods for lignocellulose hydrolysates.

Detoxification Method	Effectiveness	Disadvantages	References
Physical			
Steam stripping	Remove volatile inhibitors or inhibiting end products such as furfural and acetic acid	Ineffective in removing nonvolatile inhibitors such as lignin derivatives	Leonard and Peterson 1947; Maddox and Murray 1983; Yu et al. 1987
Evaporation			Palmqvist et al. 1996a,b; Wilson et al. 1989; Larsson et al. 1999
Solvent extraction	Remove both volatile and nonvolatile inhibitors pH dependently	Organic solvents may have detrimental effects on fermenting microorganisms. An extra step of solvent removal is necessary. Need large volumes of high-cost phase-forming polymers; poor selectivity in partition; difficulty in recovery of partitioned production, etc. High capital cost	Wilson et al. 1989; Groot et al. 1990
Aqueous two-phase extraction	Excellent biocompatibility; rapid mass transfer due to low-interfacial tension; remove both volatile and nonvolatile inhibitors; and potential for <i>in situ</i> extractive fermentation		Hahn-Hägerdal et al. 1981; Jarzebski et al. 1992; Banik et al. 2003; Hasmann et al. 2008
Supercritical fluid extraction	Excellent biocompatibility; remove furans, phenolics, and aliphatic acids polarity dependently; high concentration factor of inhibitors		Persson et al. 2002b
Encapsulation	Alleviate the inhibitory effect of toxic compounds	Complicated manipulation	Talebria and Taherzadeh 2006, 2007; Talebria et al. 2005
Molecular sieve	Remove both volatile and nonvolatile inhibitors	High sugar loss	Tran and Chambers 1986
Chemical			
Ca(OH) ₂ (overliming)	The most commonly used method; precipitate a wide range of inhibitors	Formation of gypsum; if under harsh conditions (high pH and temperature), a considerable sugar degradation occurs	Martinez et al. 2001; Persson et al. 2002a;
NH ₄ OH, NaOH, etc.	Extensive mediation and precipitation of inhibitors	Similar to overliming; NaOH is less efficient than overliming.	Sárvári Horváth et al. 2004; Alriksson et al. 2006

Table 11.1. Continued.

Detoxification Method	Effectiveness	Disadvantages	References
Reducing substances, NaHSO_3 , $\text{Na}_2\text{S}_2\text{O}_5$, KHSO_3 , Na_2S , etc. Neutralization + zeolite Diatomaceous earth Activated carbon Wood charcoal	Overcoming unfavorable oxidation-reduction potentials in hydrolysates Extensive removal of inhibitors	Less efficient than overliming Less efficient than overliming Sugar loss Need specially prepared wood charcoal and long treatment time Costly and might cause sugar loss	Leonard and Hajny 1945; Larsson et al. 1999 Eken-Saraçoglu and Arslan 2000 Ribeiro et al. 2001 Maddox and Murray 1983 Miyafuji et al. 2003
Polymeric adsorbents Mixed bed resin Ion-exchange resins	Most efficient in inhibitors removal		Weil et al. 2002 Tran and Chambers 1986 Horváth et al. 2005; Chandel et al. 2007
Biological Mutant <i>S. cerevisiae</i> Fungal isolate, <i>Coniochaeta ligniaria</i> NRRL 30616 Laccase and peroxidase from the white-rot fungus	Effective in utilizing acetic acid and keeping sugars intact Metabolizes furfural, HMF, as well as phenolics, aliphatic acids, and aldehydes Highly efficient in reducing phenolics	Not effective in reducing other inhibitors Long treatment time Not effective in reducing furans and aliphatic acids, etc.; long treatment time	Schneider 1996 López et al. 2004; Nichols et al. 2008 Jönsson et al. 1998; Larsson et al. 1999; Chandel et al. 2007
Adaptation	Makes organisms more tolerant to the inhibitors	Long treatment time and complicated manipulation	Mussatto and Roberto 2004; Martín et al. 2007; Agbogbo et al. 2008; Dinh et al. 2008

the fermentation significantly even at high concentrations. In contrast, the nonvolatile compounds severely affected both the hydrolysis and the fermentation (Palmqvist et al. 1996a,b). Palmqvist et al. (1996a) assessed the inhibitory effect of both the evaporation condensates and *nonvolatile stillage* by fermentation using *S. cerevisiae*. The most volatile fraction of a willow hemicellulose hydrolysate obtained by roto-evaporation (using a rotary evaporator) slightly decreased the ethanol productivity compared with a reference fermentation containing no volatile fraction of hemicellulose hydrolysates. But in the nonvolatile fraction obtained by roto-evaporation, the ethanol yield decreased from 0.37 g/g in the reference fermentation (glucose and nutrients) to 0.31 g/g in the treated lignocellulosic hydrolysates fermentation, and the average ethanol fermentation rate, r_{2h} , decreased from 6.3 to 2.7 g/h. As a result, commercial application of evaporation for hydrolysates detoxification may be limited.

Solvent Extraction

Solvent extraction utilizes the selective dissolving of one or more constituents of a solution into a suitable immiscible liquid solvent. It has been widely used for refining petroleum products, chemicals, vegetable oils, and vitamins. When applying solvent extraction to remove inhibitors, Wilson et al. (1989) found that ethyl acetate extraction was more effective than roto-evaporation in removing the inhibitors. The roto-evaporation removed furfural and most of the acetic acid but did not reduce lignin-derivative levels. The ethyl acetate extraction removed all the inhibitory compounds, except acetic acid, which was not completely removed by the ethyl acetate extraction process.

Aqueous Two-Phase Extraction

Aqueous two-phase systems (ATPS) are clean alternatives for traditional solvent extraction systems. ATPS are formed when two polymers, or one polymer and one salt are mixed together at appropriate concentrations and at a particular temperature. The two phases are mostly composed of water and nonvolatile polymers, thus eliminating the use of volatile organic solvents. ATPS are normally performed under mild conditions, for example, 25°C, which do not harm or denature unstable/labile biomolecules or microorganisms. In ATPS, the interfacial stress (at the interface between the two layers) is lesser (400-fold less) than that in water-organic solvent systems used for solvent extraction, causing less damage to the molecules to be extracted. The separation of the phases and the partitioning of the compounds occur rapidly. The ATPS have been tested for a number of years in biotechnological applications as a benign separation method. In addition, ATPS have been investigated for extractive fermentation (Hahn-Hägerdal et al. 1981; Jarzebski et al. 1992; Banik et al. 2003) and removal of inhibitors (Hasmann et al. 2008) from lignocellulosic hydrolysates during biofuel production from biomass.

Major disadvantages of ATPS include the relatively high cost of polymer, recycle of polymer(s), and poor selectivity, although specialized and efficient systems may be developed by varying factors such as temperature, degree of polymerization, and presence of certain ions.

Supercritical Fluid Extraction (SFE)

Any substance at a temperature and pressure above its thermodynamic critical point will become supercritical fluid, which can diffuse through solids like a gas and dissolve materials

like a liquid (Hawthorne 1990). Additionally, close to the critical point, small changes in pressure or temperature result in large changes in density, allowing many properties to be adjusted. Supercritical fluids may be suitable as a substitute for organic solvents in a range of industrial and laboratory processes.

Persson et al. (2002b) performed countercurrent flow supercritical CO₂ (200 bar and 40°C) extraction of hydrolysates. A reduction in the concentration of a variety of inhibitors, such as furan derivatives, aliphatic acid, and phenolic compounds, were observed. The effect of the SFE treatment was examined with respect to alcoholic fermentation by *Saccharomyces cerevisiae*. The ethanol yield increased from 0.30 to 0.43 g/g glucose, and its productivity from 0.14 to 0.46 g/L h. SFE has several advantages such as cleanliness, biocompatibility, and high concentration factor. But the capital cost for SFE is usually high.

Encapsulation

With cell encapsulation, fermenting yeasts are protected by an artificial membrane, and successful fermentation with toxic hydrolysates has been reported (Talebnia et al. 2005). Talebnia et al. (2005) used encapsulated *S. cerevisiae* CBS 8066 to ferment two different types of dilute-acid hydrolysates in the presence of furfural (0.39 and 0.31 g/L) and hydroxymethylfurfural (HMF; 0.74 g/L and 1.58 g/L). While the free cells were not able to ferment the hydrolysates in 24 hours, the encapsulated yeast successfully converted glucose and mannose in the hydrolysates in less than 10 hours with minimal lag phase. Talebnia and Taherzadeh (2006) further demonstrated that encapsulation is a promising method to keep the cells viable in a toxic environment and help the process to run continuously at high dilution rates and high productivities. The physiological and morphological characteristics of the encapsulated *S. cerevisiae* CBS 8066 were studied by Talebnia and Taherzadeh et al. (2007). After 20 consecutive batch cultivations in a defined synthetic medium, the ethanol yield increased from 0.43 to 0.46 g/g, while the biomass and glycerol yields decreased by 58% and 23%, respectively. The growth rate of the encapsulated cells in the first batch was 0.13/hour, but decreased gradually to 0.01/hour. After long-term application, most of the encapsulated yeast existed in the form of single and non-budding cells. Total RNA content of these yeast cells decreased by 39%, while the total protein content decreased by 24%. On the other hand, the stored carbohydrates (glycogen and trehalose) content increased. Because of the higher biomass concentrations inside capsules, the glucose diffusion rate through the membrane drastically decreased to 1/5 of that seen in cell-free capsules.

Molecular Sieve

Molecular sieves are used as adsorbents for gases and liquids. Molecular sieves have tiny pores of a precise and uniform size. Molecules that are small enough to pass through these pores will be adsorbed, while larger molecules are not. For instance, Tran and Chambers (1986) treated unfermentable red oak hydrolysates with a molecular sieve. The treatment with the molecular sieve decreased the concentration of acetic acid by 40% and furfural, by 82%. Treatment of hydrolysates with molecular sieve, however, resulted in a 10% loss in xylose concentration.

Chemical Detoxification Methods

Alkali Treatment

Alkali treatments, particularly the treatment with calcium hydroxide (overliming), have been widely used for improving the fermentability of lignocellulose hydrolysates. In an overliming process, lime is added to the hydrolysate, resulting in the formation of insoluble salts. Larsson et al. (1999) compared the effects of 12 detoxification methods, including alkali treatments with sodium hydroxide (NaOH) and calcium hydroxide ($\text{Ca}(\text{OH})_2$), on the chemical composition and fermentability of a hydrolysate from spruce pretreated with dilute acid. Overliming was more efficient than NaOH in terms of removal of inhibitors under similar conditions (Larsson et al. 1999). One drawback with overliming is the formation of calcium sulfate precipitate (gypsum). In addition, if the treatment is done at high pH and temperatures, a considerable degradation of fermentable sugars occurs (Cheng et al. 2008). The treatment has to be optimized to maximize the fermentability and lower sugar degradation.

Martinez et al. (2001) employed a titration method to predict the optimal amount of $\text{Ca}(\text{OH})_2$ for overliming at 60°C using 15 different batches of bagasse hemicellulose hydrolysate. All 15 overlimed hydrolysates exhibited the same trend, despite differences in the amount of added $\text{Ca}(\text{OH})_2$. Total furans were reduced by 51% and soluble phenolic compounds were reduced by 41%. Presumably, these furans and phenolic compounds were converted to less toxic products by overliming. Total sugars were reduced by 8.7%. Although common and effective, overliming does not remove acetic acids, which are known to inhibit ethanol production at concentrations greater than 2 g/L (Berson et al. 2006).

Ammonium ions do not form poorly soluble salts, and treatment with ammonium hydroxide compared favorably with overliming (Horváth et al. 2005). NaOH would be another option, but under similar conditions NaOH treatment has so far been less efficient than overliming. Optimal conditions were found to be in a range around pH 9.0/60°C for NH_4OH treatments and in a narrow area stretching from pH 9.0/80°C to pH 12.0/30°C for NaOH treatments (Horváth et al. 2005).

Adding Reducing Substances

Unfavorable oxidation-reduction potential (ORP) has also been cited as a cause of poor fermentability (Leonard and Hajny 1945). Collingsworth and Reid (1935) found that the addition of reducing agents to media improved their fermentability. Three methods have been proposed for overcoming unfavorable ORP in fermentation media: phytochemical reduction by large amounts of yeast; use of reducing agents; and production of reducing substances from sugars by either caramelization or alkali degradation.

When Na_2SO_3 , NaHSO_2 , $\text{Na}_3\text{SO}_3 \cdot 5\text{H}_2\text{O}$, $\text{Na}_2\text{S}_2\text{O}_3$, $\text{Na}_2\text{S}_2\text{O}_5$, KHSO_3 , Na_2S , sulfite waste liquor, alkali-decomposed sugar, ascorbic acid, cysteine, or reduced iron filings were added to wort hydrolysates, an improved fermentation was observed, which underscored the effect of ORP (Leonard and Hajny 1945; Leonard and Peterson 1947). Diethanolamine, triethanolamine, pyridine, aniline, dimethylaniline, and similar substances also showed favorable action toward fermentation under the same conditions. The amount of reducing agent required is dependent upon the length and temperature of heat treatment period (Leonard and Hajny 1945; Leonard and Peterson 1947). The mechanism of detoxification by reducing agents is not clear. However, researchers have found that toxic and oxidizing compounds such as furfural and HMF would be reduced to their less inhibitory alcohol forms inside yeast cells

associated with oxidation of NAD(P)H, and redirect yeast energy to fixing the damage caused by furans and by intracellular reduced NAD(P)H and ATP levels (Nilsson et al. 2005; Almeida et al. 2007).

Chemical Adsorption

As a physicochemical process, adsorption involves the mass transfer of a solute (adsorbate) from a fluid phase to an adsorbent surface through weak atomic and molecular forces (physical adsorption) or through weak chemical bonds (chemical adsorption). For a chemical adsorption, the adsorbate forms a monolayer on the surface of the adsorbent. Chemical adsorption methods for detoxification include the use of zeolite (Eken-Saraçoglu and Arslan 2000), eartomaceous earth (Ribeiro et al. 2001), activated charcoal (Silva and Roberto. 2001), wood charcoal (Miyafuji et al. 2003), diatomaceous earth (Ribeiro et al. 2001), polymeric adsorbents (Weil et al. 2002), mixed bed resin (Tran and Chambers 1986), and ion-exchange resins (Sárvári Horváth et al. 2004; Chandel et al. 2007).

Zeolites are widely used as ion-exchange beds in domestic and commercial water purification, softening, and other applications. Zeolites have a porous structure that can accommodate a wide variety of cations, such as Na^+ , K^+ , Ca^{2+} , Mg^{2+} , and others, which are loosely held and can readily be exchanged in a contact solution. Eken-Saraçoglu and Arslan (2000) conducted detoxification tests with CaO and combinations with zeolite during ethanol production from corn cob hemicellulose hydrolysate by *Pichia stipitis* and *Candida shehatae*. They found that the single neutralization method did not support high ethanol production (2.8 g/L) during fermentation of hydrolysates by *C. shehatae* with only 2.8 g/L ethanol obtained. However, neutralization + zeolite treatments significantly increased final ethanol concentration to approximately 6.0 g/L.

In the study of Maddox and Murray (1983), fermentation of crude hydrolysate of *Pinus radiata* by *Clostridium acetobutylicum* was not successful. After a decolorization or a steam-stripping detoxification treatment, the n-butanol fermentation was still not successful. However, a combination of the two gave a butanol concentration of up to 1.6 g/L. When activated carbon (150 g/L) was added to the hydrolysate, about 30% sugar was removed from the hydrolysates. Maddox and Murray also used anion and cation exchange resins to remove inhibitory compounds. They observed a butanol concentration of 5.7 g/L after the fermentation, which represented a yield of 17% based on sugar utilized. Wood charcoals were also tested for removal of inhibitors such as furan and phenolic compounds in wood hydrolysates (Miyafuji et al. 2003). Wood charcoals prepared at various temperatures were found to selectively remove only the inhibitors without reducing the levels of fermentable sugars. A wood charcoal treatment with a wood charcoal weight to hydrolysates ratio of 0.07 could enhance the fermentation of wood hydrolysates (Miyafuji et al. 2003). The wood charcoals prepared at higher temperatures had enhanced ability to remove inhibitors, compared with wood charcoals prepared at lower temperatures (Miyafuji et al. 2003).

Polymeric adsorbents can also be used to remove aldehydes, such as furfural, that inhibit fermentation. Weil et al. (2002) investigated the removal of furfural from a biomass hydrolysate using XAD-4 and XAD-7. The XAD-4 showed higher specificity for furfural removal than XAD-7, and it also had little interaction with glucose. The fermentation of XAD-4-treated hydrolysate with *E. coli* K011 was nearly as rapid as the control medium that was formulated with sugars of the same concentration. Liquid chromatographic analysis showed that furfural concentrations were less than 0.01 g/L compared with the initial concentrations that were in the range of 1–5 g/L. The fermentation of the resulting biomass hydrolysate also

confirmed that the concentration of furfural in the hydrolysate caused negligible inhibition (Weil et al. 2002).

Tran and Chambers (1986) compared a molecular sieve detoxification treatment with a mixed bed ion resin treatment. The molecular sieve method decreased the concentration of xylose, acetic acid, and furfural by 10%, 40%, and 82%, respectively. The mixed bed ion resin treatment, to a lesser extent, removed 7% of xylose, 20% of acetic acid, and 20% of furfural. Molecular sieve treatment produced higher ethanol (9.9 g/L) upon fermentation than mixed bed resin treatment of lignocellulosic hydrolysates (8.0 g/L).

Ion exchange resin treatment is one of most efficient methods for lignocellulosic hydrolysates detoxification (Larsson et al. 1999). Wooley et al. (1999) used weak base resins (Amberlyst A20, Rohm & Haas, Paris, France) to treat dilute-acid-pretreated poplar and reported 88% removal of acetic acid and a 100% sugar recovery. Nilvebrant et al. (2001) tested the effects of an anion exchanger (AG1-X8, BioRad Laboratories, Richmond, CA), a cation exchanger (AG50 W-X8, BioRad Laboratories), and plain resins (XAD-X8, BioRad Laboratories) on detoxification of dilute-acid-pretreated spruce and fermentation by baker's yeast. For ethanol productivity, the performance of the resins was in the sequence of AG1-X8 > XAD-X8 > AG50-X8. Sárvári Horváth et al. (2004) compared six different anion-exchange resins with different properties for detoxification of a hydrolysate from dilute-acid-pretreated spruce. The resins tested were featured by styrene-, phenol-, and acrylic-based matrices and strong as well as weak functional groups. Fractions of the hydrolysate were collected and analyzed for fermentable sugars and inhibitors, and the effect on the fermentability was evaluated using *S. cerevisiae*. An initial adsorption of glucose was found to occur in the strong alkaline environment, leading to glucose accumulation in the resin at a later stage. The fractions collected from strong anion-exchange resins with styrene-based matrices displayed the best fermentability: a sevenfold increase in ethanol productivity compared with untreated hydrolysate. The fractions from a strong anion exchanger with acrylic-based matrix and a weak exchange with phenol-based resin displayed an intermediate improvement in fermentability, a four- to fivefold increase in ethanol productivity. The fractions from two weak exchangers with styrene- and acrylic-based matrices displayed a twofold increase in the productivity. Phenolic compounds were more efficiently removed by resins with styrene- and phenol-based matrices than by resins with acrylic-based matrices. Volumetric productivity and ethanol yield increased after treatment with all six resins. The volumetric productivity in the reference fermentation, 2.2 g/L/h, was eight times higher than in the untreated hydrolysates. The volumetric productivity in the anion-exchange-treated samples was two to seven times higher than in the untreated hydrolysate. For all adsorption-based detoxification methods, the reuse or recovery of the adsorbate will determine the economics and viability of the process.

Biological Detoxification Methods

Mutant of Saccharomyces cerevisiae

Schneider (1996) used a mutant of *S. cerevisiae* YGSCD 308.3 to selectively remove acetic acid that inhibited D-xylose to ethanol conversion. The *S. cerevisiae* mutant grew on acetic acid but not on xylose, glucose, mannose, and fructose. The process reduced acetic acid to very low levels and caused only small changes in sugar concentration. The inability to phosphorylate glucose, xylose, mannose, fructose is the direct result of the presence of mutations in three genes *hxxl*, (hexokinase I), *hxx2* (hexokinase II), and *glc* (glucokinase

I). In the presence of a normally functional hexokinase II gene, for example, enzymes for the metabolism of carbon sources other than D-glucose (acetic acid and D-galactose), are repressed to low levels when D-glucose is present in the medium and vice versa when hexokinase is dysfunctional (Schneider 1996). The hydrolysate became fermentable after the treatment, and hexoses and D-xylose were subsequently converted to ethanol by the *S. cerevisiae* mutant, which might be applicable to obviation of acetic acid inhibition effects in ethanol production from hemicellulose hydrolysates.

Cellulolytic fungus Trichodenna reesei

Palmqvist et al. (1997) proposed the use of a hemicellulose hydrolysate obtained after steam pretreatment of willow for enzyme production by the cellulolytic fungus *T. reesei*. The sugars in the pentose fraction were almost completely utilized, and simultaneously the hemicellulose hydrolysate was detoxified. A reduction by more than 30% in A₂₈₀ after a 6-day fermentation indicated the degradation of phenolic components by *T. reesei*. A change in the content or structure of the phenolics may have taken place by the fungus and might as well have decreased the inhibitory action of the lignin-derived compounds. This result was confirmed by a gas chromatography-mass spectrometry (GC-MS) analysis, which showed that there was an almost complete depletion of benzoic acid derivatives during the fermentation with *T. reesei*. After fermentation, the remaining hydrolysate can be recirculated to minimize the need for freshwater. The ethanolic fermentation of the detoxified hydrolysate with *S. cerevisiae* resulted in as good and even better yields and productivities than in the fermentation containing only glucose and nutrients (Palmqvist et al. 1997). The fact that the steam-pretreated hardwood hydrolysate was detoxified by *T. reesei* could be essential for the process because the resulting enzyme-containing solution would be used to hydrolyze the cellulose fraction. The cellulase activity was 0.2 IU/mL in the *T. reesei* fermentation where no willow was added and 0.6 IU/mL where willow was added.

Fungal isolate, Coniochaeta ligniaria NRRL 30616

Lopez et al. (2004) isolated and identified new microorganisms for biological treatment of lignocellulosic hydrolysates. Several isolates with potential for abatement of inhibitors from complex fermentation substrates were obtained from soil by enrichment procedure, and selected according to their potential for depletion of toxic compounds from acid-pretreated hydrolysates. The selection was carried out in a defined mineral medium containing a mixture of ferulic acid, 5-hydroxymethylfurfural (5-HMF), and furfural as the carbon and energy sources, followed by an additional transfer into a corn stover hydrolysate (CSH) prepared using a dilute acid. Six isolates, including five bacteria related to *Methylobacterium extorquens*, *Pseudomonas* spp., *Flavobacterium indologenes*, *Acinetobacter* spp., *Arthrobacter aurescens*, and one fungus, *C. ligniaria*, were chosen based on stable growth on the above substrates. All six isolates depleted toxic compounds from the medium, but only *C. ligniaria* C8 (NRRL 30616) was effective at metabolizing furfural and 5-HMF as well as aromatic and aliphatic acids, and aldehydes (Lopez et al. 2004; Nichols et al. 2008). This strain removed 78% of 5-HMF and 97% of furfural, while overliming only decreased 51% of the total furans (Martinez et al. 2001). The possibility of including biological detoxification with *C. ligniaria* C8 in a biomass-to-ethanol conversion process with *S. cerevisiae* was tested at laboratory scale. The treatment of hydrolysate with *C. ligniaria* C8 also resulted in improved metabolism of pentoses by a recombinant bacterial strain, *E. coli* FBR5 (Nichols

et al. 2008). The *E. coli* FBR5 has a native ability to ferment glucose, xylose, and arabinose, and carries recombinant genes for selective production of ethanol. All sugars in hydrolysates treated with *C. ligniaria* C8 were consumed more quickly by the *E. coli* FBR5 than sugars in untreated hydrolysates.

Laccase and Peroxidase from the White-Rot Fungus

Jönsson et al. (1998) studied the detoxification effect of laccase, phenol oxidase, and lignin peroxidase on hydrolysates. *S. cerevisiae* was used for subsequent ethanol fermentation. The results showed more rapid consumption of glucose and a higher ethanol productivity for samples treated with laccase than for untreated samples. Treatment of hydrolysates with lignin peroxidase also resulted in improved fermentability. Analyses by GC-MS indicated that the mechanism of laccase detoxification involved removal of monoaromatic phenolic compounds present in the hydrolysate (Jönsson et al. 1998).

Chandel et al. (2007) conducted laccase detoxification tests using sugarcane bagasse hydrolysates produced by 2.5% (v/v) HCl, which contained 30.39 g/L of total reducing sugars along with various fermentation inhibitors such as furans, phenolics, and acetic acid. The laccase reduced total phenolics by 77.5% without affecting furans and acetic acid content in the hydrolysate. In comparison, the anion-exchange resin brought about a maximum reduction of 63.4% in furans and 75.8% in total phenolics, while the treatment with activated charcoal caused 38.7% and 57.5% reduction in furans and total phenolics, respectively. Fermentation of these hydrolysates with *C. shehatae* NCIM 3501 showed maximum ethanol yield (0.48 g/g) from ion exchange-treated hydrolysates, followed by activated charcoal (0.42 g/g), laccase (0.37 g/g), overliming (0.30 g/g), and neutralized hydrolysates (0.22 g/g).

Adaptation of Microorganisms

Microorganisms have the ability to adapt to perturbations of the surrounding environment to grow (Dinh et al. 2008). Utilizing the microorganism of a previous experiment as the inoculum of the next one, the adaptation of a microorganism to the hydrolysate is another biological method for improving the fermentation of lignocellulosic hydrolysate media (Mussatto and Roberto 2004). To analyze the adaptation process of *S. cerevisiae* to a high ethanol concentration, Dinh et al. (2008) performed repetitive cultivations with a stepwise increase in the ethanol concentration in the culture medium. They found that the mother cells of the adapted yeast were significantly larger than those of the non-adapted strains and that the content of palmitic acid in the ethanol-adapted strains was lower than that in the non-adapted strain in media containing ethanol.

Martín et al. (2007) adapted a xylose-utilizing genetically engineered strain of *S. cerevisiae* with sugarcane bagasse hydrolysates by 353-hour cultivation using a medium with increasing concentrations of phenols (from 1.5 to 2.3 g/L), furfural (from 0.7 to 3.4 g/L), and aliphatic acids (from 2.5 to 8.7 g/L). The performance of the adapted strain was compared with the parental strain: the ethanol yield after 24 h of fermentation of the bagasse hydrolysate with inhibitors (phenols: 1.4 g/L, furfural: 2.2 g/L, aliphatic acids: 5.0 g/L) increased from 0.18 g/g of total sugar with the non-adapted strain to 0.38 g/g with the adapted strain. The specific ethanol productivity increased from 1.15 g ethanol per gram initial biomass per hour with the non-adapted strain to 2.55 g/g/h with the adapted strain.

Agbogbo et al. (2008) investigated the effect of adaptation of *P. stipitis* in acid-pretreated CSH without detoxification for ethanol fermentation. Fermentation results showed that the

solid agar adaptation improved both the sugar consumption rate and the rate of ethanol production. Liquid and solid agar adaptation increased the sugar consumption from 64 to 72% after 96 hours of fermentation at 100 rpm. The ethanol concentration (g/L) was increased from unadapted 16.3 ± 0.51 to 18.4 ± 0.20 (liquid adapted) and 19.4 ± 0.12 (solid adapted). The solid agar-adapted strains started using xylose after 96 hours of fermentation while wild strains did not consume xylose. However, when rotation speed in the flask was increased to 150 rpm, 92% of the total sugar was consumed within 72 hours of fermentation.

Conclusion

The presence of inhibitors in lignocellulosic hydrolysates directly influences biofuel fermentation. Due to a lack of understanding about the synergistic interactions among inhibitors and the mechanisms of these interactions, highly inhibitor-resistant microorganisms might not be expected in the short term. Problems associated with biomass hydrolysates, however, may be resolved by the development of inhibitor-tolerant strains using genetic modification and metabolic engineering. From an economic standpoint, the ultimate goal is to develop a deconstruction process without detoxification. The main features of a number of detoxification methods are summarized in this chapter. Some of them are relatively new, while others have existed for decades but need some improvements for optimal performance. Among the methods, the biological detoxification methods are promising. With the isolation and development of some inhibitors degrading microorganisms and mutants, there exist some prospects of SSF of biomass to biofuel or combining a biological detoxification step with the SSF process.

Acknowledgement

This work was financially supported by Energy Biosciences Institute.

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